AN INTRODUCTION
TO THE
CHEMISTRY OF
PLANT PRODUCTS

P. HAAS
AND
T. G. HILL

Aw 34

Variation in prot. 50

crassulaceae 73

AN INTRODUCTION TO THE
CHEMISTRY OF PLANT PRODUCTS

AN INTRODUCTION

TO THE

CHEMISTRY OF PLANT PRODUCTS

VOL. II. METABOLIC PROCESSES

BY

PAUL HAAS

D.Sc., Ph.D.

READER IN PLANT CHEMISTRY IN THE UNIVERSITY OF LONDON, UNIVERSITY COLLEGE

AND

T. G. HILL

A.R.C.S., F.L.S.

READER IN VEGETABLE PHYSIOLOGY IN THE UNIVERSITY OF LONDON, UNIVERSITY COLLEGE

WITH DIAGRAMS

PREFACE.

In the preparation of the present volume on the Metabolic Processes of Plants two alternatives were presented; the one to give as full an account as possible of the literature, the other to give such an account as would form a basis for further study. The latter, and more difficult task, was chosen ; for, valuable though a digest of the relevant literature would be, it would tend to confuse rather than to assist the student. For this reason we do not profess to have mentioned all research on the subject matter ; indeed in some instances, the chapter on Growth for example, some critics will say that too much has been omitted ; we trust, however, that no work of outstanding importance and requisite for our treatment has been omitted. Details regarding methods of experiment have been omitted since this aspect of the subject more properly belongs to a practical treatise.

<div align="right">P. H.
T. G. H.</div>

July, 1922.

CONTENTS.

CONTENTS

CHAPTER I.

INTRODUCTION : THE LIVING PLANT.

THE study of plant life which in its fundamentals is physico-
chemical, is in its broad aspect the study of the origin of
life, since the plant arrived before the animal had its being.
This attitude is frankly mechanistic, to some minds grossly
materialistic, but more progress may be anticipated by follow-
ing mechanistic hypotheses than by the pursuit of theses based
on foundations the stability of which is not yet agreed on.
The present state of knowledge, however, does not permit
a full physico-chemical explanation even of phenomena ap-
parently purely chemical, and sooner or later a stage is
reached when agencies of a vitalistic nature have to be
offered in explanation.

The problems immediately involved may best be ordered
and formulated by a consideration, intentionally elementary,
of the history of a seed planted in good ground. The period
of rest completed, a period which varies much in duration
and in different species,[*] a sowed seed begins its germination
by the imbibition of water, provided the conditions, chiefly
of moisture, temperature and aeration, are suitable. When
the seed coat is saturated, water is absorbed by the under-
lying structures both by imbibition and by osmosis, for the
seed coat, although it may be impermeable to certain sub-
stances,[†] is permeable to water. Considerable swelling com-
monly results so that the volume of the seed is much increased

---

[*] The period of dormancy and consequently the beginning of germination
are conditioned by such factors as the degree of permeability of the seed coat
to water, the mechanical restraint imposed upon the embryo and associated
structures by the rigidity of the testa, the necessity of an after-ripening process
for the embryo, the degree of humidity, the amount of carbon dioxide, the
presence or absence of light, and the degree of temperature. See Crocker:
"Amer. Journ. Bot.," 1916, **3**, 99; Kühn: "Ber. deut. bot. Gesells.," 1916,
34, 369; and Crocker and Harrison: "Journ. Agric. Res.," 1918, **15**, 137.

[†] See Adrian Brown: "Ann. Bot.," 1907, **21**, 790; "Proc. Roy. Soc.,"
Lond., B. 1909, **81**, 82.

and in this swelling a relatively great force is exerted: Stephen
Hales in his classical experiment found that the force exerted
by swelling peas was sufficient to raise a weight of 184
pounds.*

The second phase in germination is now initiated, growth
starts: but growth is impossible without food to supply the
wherewithal for new structures and to make good the waste,
for vital activity requires energy which is obtained by various
oxidative processes.   Thus aerobic respiration, the ordinary
catabolic process of green plants, is a marked feature con-
current with growth and may be sufficiently intense to cause
an obvious rise in temperature.   The required food, chiefly
fats, carbohydrates and proteins, are stored in the embryo
itself or in special tissues, endosperm and perisperm: and
since the food is stored in a form mostly insoluble and
non-assimilable, water is the first essential and appropriate
enzymes the second, for not before it is hydrolized can food
be translocated from its storage cells and passed by osmotic
processes to the active tissues.   The enzymes may be
elaborated in the cells or tissues containing the food, or
may be secreted by specialized structures, the scutellum for
example.   Often the products of hydrolysis may be recognized
by simple means, sugar, for instance, in germinating barley.
In other cases their assimilation may be so rapid that
identification is difficult; indeed, sometimes their presence
can only be inferred from the results of carefully-controlled
test-tube experiments, glycerol, for example, in germinating
Ricinus.   The embryo thus presented with appropriate food,
grows and develops.   Growth is to a certain degree an
understandable problem which, on the present elementary
occasion, can be sufficiently indicated in a few words.   Of
necessity must a cell be nourished through its surface and
growth will take place if assimilation be greater than waste
by oxidative and kindred processes.   But growth means
increase, and as this increase in bulk takes place the surface
area of the cell is proportionally lessened.   A stage ultimately
will be reached when the area of the surface is so limited
in proportion to the volume of the cell as to permit the
entry of only sufficient food to make good the losses ; thus

* Hales: "Vegetable Staticks," 3rd Edition, London, 1738, p. 102.

the surface area is a limiting factor. One of three things now is possible : the cell may remain as it is, a permanent tissue element ; it may develop further, using up its own contents either entirely or in part in fitting itself for another function, water transport for instance ; or it may divide and by so doing increase its surface area in relation to its volume, in which case the cycle may restart. Growth thus can be interpreted in terms of physical chemistry : the first possibility mentioned hardly requires contemplation, since nothing is easier to do than nothing. The third proposition is less easy to understand ; the second is a mystery. For instance, why should the daughter of a merismatic cell develop into a phlœm element if it be cut off on the one side of its parent and into a xylem element if it be born on the other? Is it due to some subtle influence or stimulus which has its origin in the adjacent structural elements ; or is it due to some quality in the cell itself, an heredital predetermination ; or is it due to some obscure colloidal property comparable to the Liesegang phenomenon ? *

To these questions there are no real answers ; the facts must be accepted, their explanation must be left to the future.

The embryo grows and develops into the autotrophic organism of a form and structure determined by its conditions of life and by its ancestry and exhibiting those actions and reactions commonly associated with the higher plants. The shoots and roots circumnutate and respond to various stimuli, gravity and light being the most obvious. With respect to circumnutative and other autonomous movements, these may be explained by such conceptions as rectipetality and associated engrams ; whilst in explanation of tropisms various mechanistic hypotheses have been formulated ; some chemical, Czapek's explanation of gravitational stimulus of roots,† for instance ; others physical, the statolith theory, for example.

The highly organized root system by means of its root hairs takes up raw material by osmosis in the form of water and its dissolved salts ; in special cases, possibly in all, the osmotic strength of the cell sap of the root hairs is continuously adapted and is nicely adjusted to the osmotic strength of the soil water. From the root hairs water is passed on

* See Vol. I., p. 301.   † Vol. I., p. 361.

through the cortex to the water-conducting elements of the vascular cylinder, and this supplies the shoot system. The shoot system, no less highly organized, is, in the first instance, concerned with the manufacture of food, carbohydrate, fat and protein. In this connection the leaf, a marvel of organization —with its chlorophyll apparatus supported by the network of veins which also are the conduits for the conveyance of fluid raw materials and for the elaborated products, and with its mechanism for the regulation of gaseous interchange—is the great synthetic factory, building up food apparently with the greatest ease and certainly with remarkable rapidity.

In due season reproduction takes place. Of the problems here involved the secretion of nectar, when it obtains ; the facts of fertilization and the stimuli which invoke the segmentation of the egg ; the transmission of hereditary characters ; the reconstruction of the food destined for the use of the off-spring ; and the mechanisms of dispersal, are of fundamental importance.

Of the various aspects of the life of the plant outlined in the foregoing rather breathless account, it is appropriate on the present occasion to consider those associated with meta-bolism, the making of food and the procurement of energy. The various laws and conceptions involved have either been explained in the first volume of this work or are considered as circumstances demand in the following chapters. To this there is one exception : the determination of the concentration of the hydrogen ion is a very delicate measure of the reaction, acid or alkali, of a fluid and is invaluable in investigations where exact comparisons are required. An explanation of the principles involved is given here rather than on the occasion of its first mention in the following pages where it would unduly interrupt the narrative.

THE HYDROGEN ION CONCENTRATION.

A normal solution of any acid or salt is defined as one containing one gram of hydrogen or its equivalent dissolved in one litre of water. According to this definition, the weights of hydrochloric, nitric, acetic and any other monobasic acid contained in a litre of normal acid would be the respective molecular weights in grams, namely $HCl = 36 \cdot 5$, $HNO_3 = 63$,

$CH_3COOH = 60$.   In the case of a dibasic or tribasic acid, it would be the molecular weight divided by two or by three;

$$\frac{H_2SO_4}{2} = 49, \; \frac{H_3PO_4}{3} = 32\dot{\cdot}6.$$

From this reasoning it follows that whilst normal solutions of all these acids contain in the litre different quantities of acid, they all contain the same quantity of hydrogen, namely, 1 gram per litre.   Whilst, however, they all contain potentially the same amount of hydrogen, it does not follow that the whole of this quantity is ionized ; and inasmuch as the actual acidity of

ERRATA.

P. 5, line 12 from bottom, *for* 0·1N *read* normal.

P. 6. The calculation of the hydrogen ion concentration of decinormal hydrochloric acid should have been made for 91 per cent instead of 97 per cent which would have given $P_H = 1\cdot04$ instead of $P_H = 1\cdot01$.

As a matter of fact, hydrochloric acid, which is a strong acid, is almost entirely ionized in dilute solutions, whilst acetic acid in solutions of equivalent strengths is ionized to a much smaller degree.   In actual figures, about 97 per cent. of the hydrogen in a ·001N solution of hydrochloric acid is ionized and only about 84 per cent. in a 0·1N solution, whilst in ·001N acetic acid not more than 13·6 per cent. of the hydrogen is ionized. Thus in the case of these two acids of the same normality, although the total amount of titratable hydrogen, as determined by the alkali-neutralizing power, is found to be the same, the actual percentage of ionized hydrogen is seven times as great in the case of the hydrochloric as in that of the acetic acid.

That the comparatively feebly ionized acetic acid ultimately requires the same amount of alkali for neutralization as the more strongly ionized hydrochloric, is due to the fact that as the ionized hydrogens in the acetic acid are neutralized a fresh quantity of previously un-ionized hydrogens become ionized to

take the place of those which have been neutralized, and so on until all have been satisfied.

In practice it is the ionized hydrogen only which is responsible for the acidity of a solution at any given moment and so it comes about that the hydrogen ion concentration for a solution is, for biochemical purposes, a much more valuable criterion of the actual conditions prevailing in any given circumstances than is the potential alkali neutralizing power.

The concentration of hydrogen ions may be expressed as follows. In a decinormal solution of hydrochloric acid there would be 0·1 gram in 1000 c.c., presuming it to be completely ionized. In actual fact, however, a decinormal solution of hydrochloric acid is only ionized to the extent of 97 per cent., consequently the concentration is only $0·1 \times 97$ or $9·7 \times 10^{-2}$. This concentration is more conveniently expressed as a logarithm : $\log_{10} 9·7 = ·9868$, wherefore $9·7 \times 10^{-2} = 10^{·9868-2} = 10^{-1·01}$. It has been agreed to express hydrogen ion concentration as the exponent to the base 10 of the concentration with the negative sign omitted, and this is represented by the symbol P_H. Hence the hydrogen ion concentration of the above $N/10$ hydrochloric acid would be $P_H = 1·01$; if completely ionized it would be $P_H = 1$.

On this principle the following are synonymous methods of expression :

$$N = \frac{N}{10^0} = P_H 0 \qquad \frac{N}{1,000,000} = N \times 10^{-6} = P_H 6$$

$$\frac{N}{10} = N \times 10^{-1} = P_H 1 \qquad \frac{N}{10,000,000} = N \times 10^{-7} = P_H 7$$

$$\frac{N}{100} = N \times 10^{-2} = P_H 2 \qquad \frac{N}{100,000,000} = N \times 10^{-8} = P_H 8$$

$$\frac{N}{1000} = N \times 10^{-3} = P_H 3 \qquad \frac{N}{1,000,000,000} = N \times 10^{-9} = P_H 9$$

$$\frac{N}{10,000} = N \times 10^{-4} = P_H 4 \qquad \frac{N}{10,000,000,000} = N \times 10^{-10} = P_H 10$$

$$\frac{N}{100,000} = N \times 10^{-5} = P_H 5$$

It will be seen from the above that the greater the value of P_H, the lower is the actual hydrion concentration. Moreover, it is an established fact that the product of the concentrations of the hydrogen and hydroxl ions in any given solution, is a constant, namely—

$$C_H \times C_{OH} = 10^{-14·14}$$

and consequently at exact neutrality, when the concentrations of the two are exactly equal, $C_H = 10^{-7.07}$ and $C_{OH} = 10^{-7.07}$ whence it follows that for absolute neutrality, in which the concentration of hydrogen ions is exactly equal to that of the hydroxyl ions $P_H = 7.07$.

There is no need to determine the OH ion concentration since it is easily found from the difference between 14.14 and the hydrion concentration.

Thus for $P_H 1$ the hydroxyl ion concentration would be $P_{OH} 13.14$, and for $P_H 10$ it would be $P_{OH} 4.14$.

Since for $P_H 7.07$ there is exact equality between H and OH ions, it follows that on either side of this value one or other will be in excess. Thus values of P_H below 7.07 indicate acid solutions, while values of P_H above 7.07 are alkaline.

The most accurate method of determining P_H is the electrical method depending upon conductivity determinations. For practical purposes, however, a colorimetric method has been devised depending upon the fact that a series of indicators have been found whose colours depend upon the prevailing P^H and which are sensitive to changes in P_H within certain well defined limits.

Taking for example the commonly used indicators, the range for methyl orange is from $P_H 3.1$ to $P_H 4.4$ red to yellow.

litmus $P_H 5.4$ to $P_H 7.8$ red to blue.
phenolphthalein $P_H 8.3$ to $P_H 10$ colourless to red.

It will be seen from this table that owing to the fact that these indicators each have their clearly defined range of sensitiveness, it follows that one and the same liquid, such as urine, with a $P_H 5$ may have an alkaline reaction to methyl orange and yet be acid to litmus or phenol phthalein, and for the same reason a solution which is neutral to litmus may still be acid to phenolphthalein. This is well illustrated by the fact that many media which require to be neutralized previous to use require more alkali for neutralization if phenol-phthalein is used as indicator than if litmus be employed.

Within recent years the importance of hydrogen ion concentration to the well being and growth of plants has been more and more recognized.

In most living organisms provision is made for securing that the P_H of the medium shall not be easily disturbed ; this

is effected by the presence of certain salts such as the phosphates of the alkali metals or sodium bicarbonate, etc. These salts exert what is known as a Buffer action in counter-acting any considerable increase in P_H on the introduction into the solution of a small quantity of acid. This principle may be illustrated as follows. If a single drop of dilute hydrochloric acid is added to a quantity of distilled water, the P_H of this water, which should be 7·07, may be very considerably altered, and the same would apply if instead of pure water, a dilute solution of sodium chloride had been used. If, however, the water had contained, in the place of the sodium chloride, an equivalent amount of sodium phosphate, the effect of the addition of the hydrochloric acid would merely have been to displace a corresponding amount of feebly ionized phosphoric acid whereby the P_H would have been hardly altered at all. This may be expressed by saying that sodium chloride has no buffer action whereas sodium phosphate and the salts of other feeble acids, such as boric, citric and amino acids, have strong buffer action.

The blood, as a typical physiological fluid, is provided with a complex system of sodium phosphate and bicarbonate which has a most efficient buffer action preventing the fluid from having its P_H appreciably altered in the event of the sudden abnormal development of acid.

Acting upon this principle, standard solutions of known P_H are best made from suitable concentrations of salts of known marked buffer action; such solutions may be kept without fear of alteration through contamination with atmospheric car-bon dioxide or alkali from the glass bottle, whereas solutions made from salts with little or no buffer action would rapidly alter and be useless.

In practise it is found convenient to keep a number of such standard buffer solutions of known P_H for the purpose of determining the P_H of a given liquid by comparison of the colours given with the same indicator. For this purpose a small quantity of the liquid under examination is treated with a few drops of the appropriate indicator and its colour is matched against that buffer solution which gives the closest approximation to its own with the same indicator. It should be noted that the indicators employed in this work are sensitive

only over a certain range of P_H, say from $P_H 2 \cdot 8$ to $P_H 4 \cdot 6$ for bromphenol blue and from $P_H 4 \cdot 4$ to $P_H 6 \cdot 0$ for methyl red, and from $P_H 6$ to $P_H 7 \cdot 6$ for bromthymol blue and so on ; hence if no match was obtained with one indicator, the P_H of the solution lies outside that range and another indicator has to be employed until the correct one has been found and the P_H fixed with the greatest possible degree of accuracy. With a little practise it becomes possible to detect differences of $0 \cdot 1$ in the value of the P_H.

The applications of the above principles to the problems of soil chemistry will be obvious, and it will be realised that some of the soil constituents have marked buffer action.

CHAPTER II.

THE SYNTHESIS OF FATS.

IN the plant fats are commonly associated with the reserve
food in seeds, spores, and vegetative perennating organs. As
a food they have considerable value in that on physiological
combustion they yield more energy weight for weight than
either protein or carbohydrate, the relative energy producing
values being roughly 5 : 3 : 2 respectively.

A consideration of the salient features regarding the physio-
logical significance of fats has been given in the first volume,*
in which place the consideration was retained for the sake of
a closer association of the characterization and other features
of fats with the physiological problems involved. For this
reason a general survey only, with such departures into detail
as necessity demands, will be given on the present occasion.

The fat characteristic of a plant makes its appearance in
the storage organ as that member approaches maturity. The
problem immediately presented is the origin of the fat. There
can be but little doubt that it is formed *in situ* from materials
in the immediate neighbourhood, a fact which is emphasized
by many observations on the development of fats in seeds
isolated from the plant whilst in an immature condition and
originally containing little or no fat.

Within the storage organ, the fat may have its origin in
the products of the hydrolysis of fat synthesized in some other
organ, the leaf for example, in much the same way as the
starch in the potato tuber has its ultimate origin in the carbo-
hydrate produced in the leaf. Possibly the best ascertained
instance of the normal occurrence of fat, or fat-like substances,
in green assimilating organs is that provided by *Vaucheria*.

With regard to the higher plants, the facts relating to the
occurrence of fats and kindred substances in the leaves do not

* Vol. I., p. 36. See also Terroine : " Ann. Sci. Nat. Bot.," 1919, sér. x. 1, i.

permit the drawing of a definite conclusion in regard to the present problem, and, in fact, there would appear to be no evidence to warrant the conclusion that fats have a synthetic origin from raw materials in the same sense as carbohydrates. For this reason their origin must be sought out elsewhere : the proteins, the carbohydrates, and glycerol and fatty acid are amongst the more obvious sources.

With regard to the proteins, evidence is not wanting that they, under certain conditions, may be converted into fats ; but the evidence relates to the animal rather than to the plant.

WALNUT.

Date of Gathering.	Per Cent Fat.	Per Cent Glucose.	Per Cent Saccharose.
6 July	3	7·6	0
1 August . . .	16	2·4	0·5
15 August . . .	42	0	0·6
1 September . .	59	0	0·8
4 October . . .	62	0	1·6

ALMOND.

Date of Gathering.	Per Cent Fat.	Per Cent Glucose.	Per Cent Saccharose.	Per Cent Starch and Dextrins.
9 June . . .	2	6	6·7	21·6
4 July . . .	10	4·2	4·9	14·1
1 August . .	37	0	2·8	6·2
1 September . .	44	0	2·6	5·4
4 October . .	46	0	2·5	5·3

With respect to the carbohydrates, the weight of opinion favours the view that in these substances is to be found the origin of fats.

The conversion of carbohydrate to fat in plants, or parts of plants, exposed to a low temperature ; the development of fats in immature seed separated from the parent plant ; and the fattening of animals on a carbohydrate diet indicate the close physiological connection of fats with carbohydrates.

Quantitative results are not wanting. Numerous observations, amongst which those of Schmidt, Le Clerc du Sablon, de Luca, Funaro, and Ivanow may be mentioned, show that in the maturation of the seed, the increase in the amount of

fat and the decrease in the amount of carbohydrate are con-current. This is illustrated in the above tables representing the results of two experiments by Le Clerc du Sablon.

The carbohydrates which serve as raw material for the elaboration of fats vary with the plant; glucose, sucrose and starch would appear to be most commonly employed in this connection, the last two being initially hydrolysed.

It is a well-known fact that carbohydrates appear during the germination of fat containing seeds. Thus Mazé * found that the isolated cotyledons of arachis seeds increased in their dry weight from 2·2613 to 2·6153 grams, an increase due to the fixation of oxygen, whilst their sugar content increased from ·3416 to ·4684 grams.

The work of Le Clerc du Sablon, Ivanow, Miller and others have thrown much light on the hydrolysis of fats in their studies on the germination of various fat-containing fruits and seeds. Lipase is the enzyme concerned and by its activity the fat is hydrolysed into glycerol and fatty acid, which products are used either immediately for the needs of the seedling or are first converted into carbohydrate. Agreement is general that as germination proceeds, the acid radicles become saturated, as is indicated by the decrease in the iodine value, and the rate of consumption of these acids is inversely proportional to their degree of saturation.

As the fats decrease, the carbohydrates increase, a fact brought out in the following analysis of arachis seedlings by Maquenne.†

Age in Days.	Per Cent Fat.	Per Cent Carbo-hydrate other than Cellulose.	Cellulose and other Insoluble Carbohydrates.
0	51·39	11·55	2·51
6	49·81	8·35	3·46
10	36·19	11·09	5·01
12	29·00	12·52	5·22
18	20·45	12·34	7·29
28	12·16	9·46	9·48

The nature of the carbohydrate formed differs in different plants; glucose would appear to be the most common, but

* Mazé : " Compt. rend.," 1900, **130**, 424.
† Maquenne : " Compt. rend.," 1898, **127**, 625.

saccharose, starch and dextrins also are described. It is not unlikely, as Maquenne has pointed out, that the nature of the carbohydrate depends upon the kind of fat and its degree of saturation. He considers that the saturated fatty acids contribute in a much lesser degree to the formation of sugars than do the unsaturated acids, and that the saturated fatty acids are principally used for respiratory purposes. Agreement, however, between the authorities in respect to this aspect of the subject does not obtain. Ivanow considers that there is no real difference between the saturated and unsaturated fatty acids in their power to give origin to carbohydrates. The difference in their amounts is due to the more rapid conversion of the unsaturated variety. However this may be, the salient feature in the germination of a fat-containing seed is the conversion of the fat into carbohydrate, the reverse to what obtains during the maturation of the seed. The change is effected by the activity of lipase which hydrolyses the fat into glycerol and fatty acid.

The work of Ivanow has shown that lipase has a reversible action, and the fact whether it hydrolyses or synthesizes fats is merely a question of conditions, mainly the presence or absence of water. The glycerol extract of a fat-containing seed, which extract contains the lipase, mixed with oleic acid will synthesize a fat: the addition of water will result in the hydrolysis of this fat into glycerol and fatty acid.

In the synthesis of fats, Ivanow considers that higher saturated acids of the fatty series are the first to be formed from the sugar, and these are converted into unsaturated acids which combine with the glycerol to form the fat.

With regard to the origin of glycerol, the chemical relationship between this substance and glucose is so close as to suggest at once the possible inter-relation of the two; further, glycerol may have an origin in respiratory processes as is shown by the production of this substance during the alcoholic fermentation of sugar.

CHAPTER III.

THE SYNTHESIS OF CARBOHYDRATES.

THE term carbon assimilation, although unfortunate from some points of view, is employed to designate all those activities, in part physical, in part chemical, which play a rôle in the anabolism of carbon dioxide by green tissues. The conspicuous facts of the process are that active chlorenchyma on exposure to light forms, by means of its chlorophyll, carbohydrate from the initial substances carbon dioxide and water; oxygen, in volume roughly equivalent to the volume of carbon dioxide consumed, is evolved during the process.[*] Carbohydrate is the obvious and chief end product, but protein also may be so formed, and such diverse materials as fat, tannin and various organic acids have been considered, probably on insufficient evidence, to be of direct photosynthetic origin. The earlier phases in these synthetic processes are photochemical, a mutation of radiant into chemical energy, and it is during this phase that the oxygen, a waste product, is evolved. The presence of oxygen in the air-space system of the active chlorenchyma may thus be considerably greater than in normal air, and since this gas is continually excreted during the process, it is not surprising to find that the quantity of oxygen in the surrounding atmosphere is immaterial to the process and that it may be decreased to 2 per cent or increased to 50 per cent without adverse effect : [†] but since

[*] Bonnier and Mangin (" Ann. Sci. Nat. Bot.," 1886, **3**, 1) found by various experimental methods that the ratio O_2/CO_2 was always greater than unity for ordinary plants; the lilac gave the smallest value 1·05, and the holly the largest 1·24. A similar range was found by Aubert (" Rev. gén. Bot.," 1892, **4**, 203) to obtain in ordinary plants, but succulents, which have a peculiar metabolism, gave generally a higher value; as high as 7·59 in the instance of *Opuntia tomentosa*. Maquenne and Demoussy (" Compt. rend.," 1913, **156**, 506) conclude from a large number of observations that the assimilatory quotient approximates to unity.

[†] Friedel: " U.S. Dept. Agric.," 1901, Bull. 28.

the formation of chlorophyll is dependent upon the presence of oxygen, the prolonged maintenance of a low oxygen pressure will inhibit the development of more chlorophyll and this will in turn react on carbon assimilation.

Carbon assimilation is conditioned by various factors ; wherefore the rate of the process will vary considerably according to the inter-relationships of these factors. For this reason it is hardly remarkable to find that different values of the rate of carbon assimilation have been reported by different investigators : differences in value due not only to inappreciation of the conditioning factors, but also to different avenues of attack and to experimental error.

The accompanying table gives a selection of values of the rate of carbon assimilation in the open air of detached leaves of the sunflower, *Helianthus annuus*, expressed in terms of grams of increase in dry weight per square decimeter per hour, obtained by the authorities named :—

Sachs * . . .	·01882
Brown and Morris † .	·00985
Brown and Escombe ‡	·00361 – ·00551
Thoday § . . .	·0169 (the average for fully turgid leaves) –
	·0016 (the average for quite flaccid leaves).

The figures of Sachs and Thoday were obtained by the direct determination of the increase in dry weight, the well-known method of Sachs, whilst the others were calculated from the amount of carbon dioxide absorbed and assuming that carbohydrate only was ultimately formed. Since the ultimate fate of the carbon dioxide is not entirely known, the extent to which it is directly used in the elaboration of fat or protein for example, the dry weight method would appear to give the most accurate results.‖

THE FACTORS.

It is obvious that little or no profit will accrue from the contemplation of the above figures unless they be correlated with the factors which determine and control the process.

* Sachs : " Arbeit. Bot. Inst.," Würzburg, 1884, **3**, 19.
† Brown and Morris : " Journ. Chem. Soc.," Lond., 1893, **63**, 604.
‡ Brown and Escombe : " Proc. Roy. Soc.," Lond., B. 1905, **76**, 29.
§ Thoday : *Id.*, 1910, **82**, 421.
‖ For a critical review of Sachs's and Brown and Escombe's methods, see Thoday : " Proc. Roy. Soc.," Lond., B. 1909, **82**, 1.

The doctrine of limiting factors, now well known, is due to F. F. Blackman * who enunciated the axiom that when a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the slowest factor. The limiting factor in any definite instance may be identified by the experimental application of the principle that "when the magnitude of a function is limited by one of a set of possible factors, increase of that factor, and of that one alone, will be found to bring about an increase of the function." †

The principle may be illustrated by one of Matthaei's ‡ many experiments on the effect of temperature on carbon assimilation under conditions constant except for temperature and illumination. In the case of *Prunus laurocerasus* there was a gradual increase in the assimilation as the temperature was raised; at about 11° C. a maximal assimilation of 22 mg. of carbon dioxide per 50 square cm. per hour obtained and was not increased even by raising the temperature to 25° C. By doubling the light intensity, however, the maximal assimilation was equivalent to 37·5 mg. of carbon dioxide per 50 square cm. per hour and again there was no increase on raising the temperature. This means that light intensity was a limiting factor and only by its increase could a greater carbon assimilation be obtained.

The factors which condition carbon assimilation are external and internal : the external factors are capable of control whilst the internal are much less amenable to experiment and thus are less understood. Of the external factors, the raw materials, temperature, and illumination are the most conspicuous ; and of the internal factors, chlorophyll and the products of carbon assimilation are the best understood.

* Blackman : "Ann. Bot.," 1905, **19**, 281.

† Blackman and Smith : " Proc. Roy. Soc.," Lond., B. 1911, **83**, 389.

‡ Matthaei : " Phil. Trans. Roy. Soc.," Lond., B. 1904, **197**, 47.   For the application of the principle to the growth of field crops see Balls and Holton : " Phil. Trans. Roy. Soc.," Lond., B. 1915, **206**, 103, 403 ; and Balls : *Id.*, 1917, **208**, 157.

EXTERNAL FACTORS.

THE RAW MATERIALS.

Water and carbon dioxide are the requisite raw materials for carbon assimilation.

WATER.—Water is essential not only as such for the fabrication of food, but also to keep the leaf tissues in a condition mechanically fit for the processes to take place. Thoday found that the rate of carbon assimilation lessened as the leaves of *Helianthus annuus* lost their turgidity ; in an extreme instance, when the leaves were very flaccid, the stomates were all but closed and the increase in dry weight was very small indeed. Some determinations by Thoday of the average increase in dry weight of leaves in different conditions of turgidity have been mentioned. If cells become plasmolysed, constructive activities must cease ; if in such cells the turgid condition be not recovered, death supervenes. With regard to the water supply, the transpiration current is the immediate source ; it is, however, not convenient on the present occasion to consider the problems presented by this phenomenon.

CARBON DIOXIDE.—Under normal conditions, the carbon dioxide for carbon assimilation is derived from the atmosphere and to a lesser extent from the products of respiration.* The amount of respiratory carbon dioxide is conditioned mainly by the temperature and may be equal to half the possible inflow from the atmosphere at the higher temperatures possible in laboratory experiments. The entry of atmospheric carbon dioxide into the plant is either through the intact epidermis, as in those plants which like certain aquatics lack stomates, or mainly through the stomates and, to a much lesser and negligible extent, provided the amount of carbon dioxide is not unduly increased, through the unbroken epidermis. This cuticular path of gaseous interchange once was thought to be the

* Under abnormal conditions it appears that plants can make use of carbon dioxide from the soil. Pollacci ("Atti. Inst. Bot. Univ. Pavia," 1917, **17**, 30) found that plants grown in soil rich in humus or in water culture enriched with carbon dioxide could form starch and increase in dry weight notwithstanding the fact that their aerial parts were in an atmosphere freed from carbon dioxide. The assimilation, however, was not sufficient for normal growth.

main route, but the work of F. F. Blackman [*] and of Brown
and Escombe [†] has shown that the stomates are the important
paths.   F. F. Blackman found by direct measurement that the
degree of gaseous interchange was proportionate to the distri-
bution of the stomates, results which Brown and Escombe con-
firmed in respect to plants with stomates on but one surface
of the leaf, but with regard to instances in which stomates
occur on both surfaces of the leaf, they found that in bright
sunlight the intake of carbon dioxide into the upper surface
is greater than would be expected from the ratio of distribu-
tion of the stomates on the two sides; in light of a lesser
intensity, however, there is a closer, but not very close, corre-
spondence between the intake of carbon dioxide and the pro-
portional distribution of the stomates.   It is suggested [‡] that
the greater infusion found to obtain into the upper side of
amphistomatous leaves may be accounted for in that partial
opening of the stomates is likely when the incidence of illumi-
nation is on that side, and that since the palisade parenchyma
is the more active part of the mesophyll, there will be a steeper
diffusion gradient in the upper side which will promote a more
rapid flow of carbon dioxide through the stomates of the upper
surface.

The movements of the carbon dioxide are in accordance
with the laws of gaseous diffusion; the pressure of carbon
dioxide in the active chlorenchyma will be very low, whilst in
the atmosphere surrounding the leaf it will correspond to, say,
three parts in 10,000.   Thus there are set up diffusion cur-
rents the gradients of which vary according to the conditions,
rate of use and degree of atmospheric motion for example,
obtaining.   The problem of interchange between the gases
contained in the leaf and in the surrounding atmosphere is
not, however, so simple as may appear from this statement.
Brown and Escombe, experimenting with leaves of *Catalpa
bignonioides*, found that the rate of absorption of carbon
dioxide at normal temperature and pressure was about 0·07
c.c. per sq. cm. per hour; since the total area of the stomates
was but 0·9 per cent of the total leaf surface, it follows that

[*] Blackman: " Phil. Trans. Roy. Soc.," Lond., B. 1895, **186**, 485, 503.
[†] Brown and Escombe: *Id.*, 1900, **193**, 223.
[‡] *Ibid.*: " Proc. Roy. Soc.," Lond., B. 1905, **76**, 29.

carbon dioxide must pass through the openings at the rate of 7·77 c.c. per sq. cm. per hour, an amount so considerable when regard is had to the stomatal area and to the fact that this rate of absorption is about fifty times greater than the absorption of atmospheric carbon dioxide by a normal solution of caustic potash, that it is hardly surprising that earlier physiologists laid much stress on cuticular gaseous interchange.

The conditions obtaining in an active green leaf are briefly these : the active chlorenchyma is absorbing carbon dioxide, which must enter the cell in a state of solution, from the air-space system on which it abuts ; hence the pressure of carbon dioxide in the immediate neighbourhood of these surfaces of absorption will have a very low value, possibly approximating to zero under ideal conditions.   Diffusion currents are thus set up, a falling gradient of carbon dioxide density from the "respiratory chamber" of the stomatal apparatus obtaining. Renewal of carbon dioxide is from the external air through the pore of the stomate, a cylinder of a certain length and, in the simplest cases, of approximately uniform diameter when the guard cells are fully turgid.   Clearly various tensions of carbon dioxide occur : the maximum in the atmosphere, theoretically at an infinite distance from the leaf but practically at a distance equivalent to five or six times the diameter of the stomate, and the minimum at the absorbing surfaces of chlorenchyma ; thus a gradient of density of carbon dioxide is formed so that a drift of this gas from the outer atmosphere to the chlorenchyma obtains.   The path followed by the carbon dioxide is obstructed by the stomates and may be divided into three sections : from the remote atmosphere where the pressure of carbon dioxide is greatest, ρ, to the outer opening of the stomate ; through the shaft of the stomate when the pressure of carbon dioxide is less, ρ' ; and from the inner opening of the stomatal shaft to the surfaces of absorption where the pressure of carbon dioxide is, ideally, zero.   The interposition of the stomata exercises a profound influence on this diffusion.   Brown and Escombe [*] demonstrated that the rate of diffusion through such an absorbing disc as is represented by a stomate is proportional to

* Loc. cit.

the diameter of the opening ; * they visualize zones of equal density above the stomate varying from the atmospheric density, ρ, to a lower density, ρ', at the stomate cut perpendicularly by the lines of flow of carbon dioxide converging to the opening of the stomate † (Fig. 1). Thus it is that the increased flow of gas through the stomate is possible.

The second section in the route of the carbon dioxide is through the tube formed by the guard cells. Through this tube the flow is inversely proportional to the length of the tube, but the system of external shells increases the resistance to the flow (Fig. 2). Finally, in the third section the tube
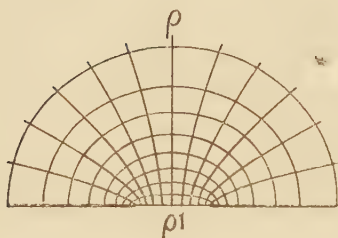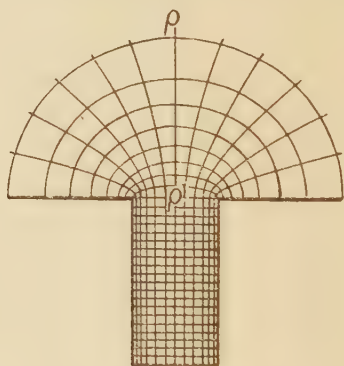

FIG. 1.   FIG. 2.

opens into the air space system of the mesophyll bounded by the absorbing surfaces of the chlorenchyma ; here the converse of the first part of the path obtains, diffusion shells over the lower opening of the stomate, where the density of the carbon dioxide is ρ', being formed (Fig. 3). Thus in the whole system there is a gradient of density from ρ to, say, o, with a set of shells at either end of the stomatal tube (Fig. 4).

The obstruction to gaseous diffusion inseparable from a multiperforate septum such as the stomatal epidermis of a leaf, varies according to the distance apart of the perforations : if they are placed at distances roughly equal to ten times the

* Larmor's Law of Diameters :—$Q = 2k\rho D$ where Q = amount of CO_2 absorbed in a given time; k = cœfficient of CO_2 in air; ρ = density of atmospheric CO_2 at a point far removed from the absorbing disc ; D = diameter of disc.

† $Q = 2 k (\rho - \rho') D$.

diameter of a perforation, each will act independently without interference by its neighbours and conform to the law of diameters. When situated more closely together, it was found that the obstacle to diffusion was much less than the actual obstruction of area by the solid portions of the septum. To quote concrete examples, Brown and Escombe calculated that in *Helianthus* the leaves could absorb 2·578 c.c. of carbon dioxide per sq. cm. per hour in moving air and 2·095 c.c. per sq. cm. per hour in still air, assuming that the stomates are
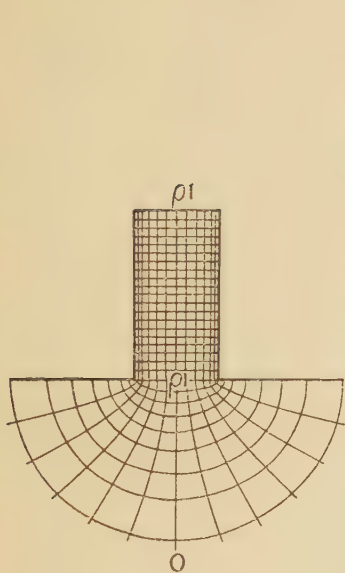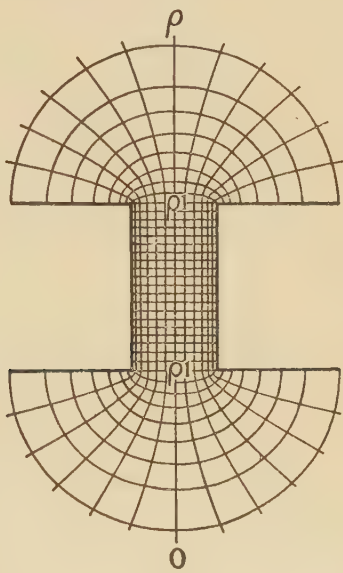

FIG. 3.   FIG. 4.

circular in shape. By actual measurement it was found that in this same plant * in diffused light at a temperature of 19° C., 0·434 c.c. of carbon dioxide, per sq. cm. per hour was absorbed, an amount much less than the capacity of the stomates to supply. Those conditions, humidity of atmosphere, temperature and illumination, which affect the size of the stomates obviously will affect the infusion rate of carbon dioxide.† Under natural conditions carbon assimilation is limited by the

* Brown and Escombe: "Proc. Roy. Soc.," Lond., B. 1905, **76**, 29.

† See Darwin: "Phil. Trans. Roy. Soc.," Lond., B. 1898, **190**, 531; 1916, **207**, 413. Knight: "Ann. Bot.," 1916, **30**, 57.

low pressure of carbon dioxide in the atmosphere, the high values obtained in experiments under conditions involving an increased supply of the gas never being attained.

In experimental work with land plants a limit is set to the increase of carbon dioxide supply by the narcotic effect of the gas when in excess,* 25 per cent generally will inhibit growth ; aquatic plants, on the other hand, are able to withstand a relatively high concentration.   Blackman and Smith † found that *Fontinalis* and *Elodea* in water with a carbon dioxide concentration of 33·92 and 35·82 per cent of saturation, and under identical intensity of illumination and at temperatures of 23° C. and 28° C. respectively, assimilated ·0223 and ·0249 grams of carbon dioxide per hour per standard area of 137 sq. cm. *Fontinalis* is less efficient, for reasons not finally determined, than aquatic angiosperms such as *Elodea*, *Potamogeton* and *Ceratophyllum* in utilizing carbon dioxide.   Blackman and Smith, experimenting with *Elodea* and *Fontinalis* under constant conditions of light and temperature and with a carbon dioxide supply ranging from ·0025 to ·0540 gms. per 100 c.c. of water, found that the carbon assimilation increases steadily in proportion to the increase in the supply of carbon dioxide. When the assimilation reaches about ·023 grams of carbon dioxide per hour, however, there is no further increase with an augmented carbon dioxide unless the light intensity be increased.   In other words, light intensity in this connection is a limiting factor.

The difficulties inseparable from critical investigations in the field are obvious : of recent work, that of McLean ‡ on the carbon dioxide absorption of coconut leaves under natural conditions may be mentioned.   He found that the rate of absorption is at a maximum in the morning, a depression obtains at mid-day followed by an increase in the afternoon and then a final decline towards sunset.   Similar values were obtained for detached leaves, but the curve showed a single maximum at about noon instead of two maxima which normally obtain with attached leaves, for which difference there is no adequate explanation.   Clearly some internal

---

* See Chapin : "Flora," 1902, **91**, 348.

† Blackman and Smith : "Proc. Roy. Soc." Lond. B. 1911, **83**, 389.

‡ McLean : "Ann. Bot." 1920, **34**, 367.

limiting factor is operating, possibly connected with the accumulation of the products of carbon assimilation (p. 35). It was also found that immature and old leaves absorbed carbon dioxide at a lesser rate than leaves of an intermediate age.

TEMPERATURE.

The statement that chemical change is profoundly influenced by temperature needs no elaboration : in the majority of instances an increased temperature accelerates a reaction, examples in which the contrary obtains are very few. Van't Hoff's Law states that for every rise in temperature of 10° C. the reaction is increased at a definite rate, in general terms doubled or trebled, the precise value of which is specific to the reaction.* The plant, however, is not a test-tube but a very complex system of reacting substances, wherefore it is only in experiments most carefully controlled and skilfully conducted that approaches to the mathematical preciseness of well ascertained physico-chemical laws will obtain.

Long has it been known that an increased temperature results in an increased carbon assimilation, but it was not before F. F. Blackman's experimental researches on vegetable assimilation and respiration that the subject was really critically examined. Matthæi † at the outset of her work on the effect of temperature on carbon assimilation found that in addition to the influential external conditions there is an important internal condition of a plant or plant member, the result of previous treatment such as excess of food, starvation, and change in temperature. This has a most important bearing on experimental results, a fact previously overlooked, so that in order to have comparable results it is essential that the previous history of all the material employed should be the same.

In the case of *Prunus laurocerasus* it was found that for each temperature to which the leaves were subjected there is a definite amount of carbon assimilation, the maximal assimilation for that temperature, which cannot be exceeded and

* This factor is termed the temperature coefficient and is represented by the symbol K with a number attached indicating the number of degrees concerned, e.g. K_{10}.

† Matthæi : " Phil. Trans. Roy. Soc.," Lond., B. 1904, **197,** 47.

cannot be attained unless the illumination be of sufficient intensity and the carbon dioxide be adequate in amount.
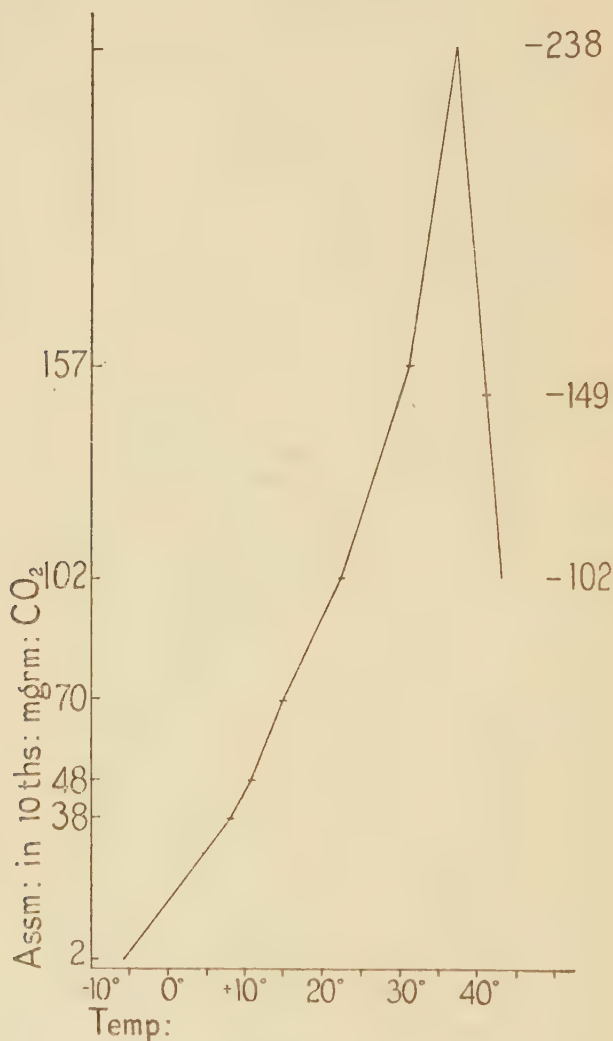

FIG. 5.

These maximal amounts of assimilation increase rapidly with rising temperatures but at the higher temperatures the initial rate for that temperature cannot be maintained for long but

falls off regularly at a rate dependent on the temperature; the higher the temperature the quicker the fall, which rapidity, however, is not maintained. Thus there is a time factor for the higher temperatures. Fig. 5 summarizes the results obtained by Matthæi; it will be seen that the turning point is $37 \cdot 5°$ C. which was found to be within a few degrees of the temperature fatal to the leaf.

The results obtained for temperatures below 25° C. conformed to Van't Hoff's Law, the coefficient of increase in the rate of carbon assimilation for a rise of 10° C. being $2 \cdot 1$. In subsequent investigations Blackman and Matthæi found the assimilation coefficient for the leaves of *Helianthus tuberosus* to be $K_{10} = 2 \cdot 3$, whilst for *Elodea*, $K_{10} = 2 \cdot 05$.* The culminative point of the assimilatory curve (Fig. 5) in respect to increasing temperature is important and is paralleled in enzyme action; in view of the results obtained by Willstätter and Stoll, it is not unlikely that the inhibition or destruction of an enzyme at these higher temperatures may be a limiting factor.

With regard to the internal temperature of assimilating leaves, this will vary considerably in accordance with such conditions as the intensity and quality of the light, the character of the leaf surface and so on; Blackman and Matthæi † demonstrated, by thermoelectric means, an excess of 7° C. to 16° C. in the leaves of *Prunus laurocerasus* in bright sunlight above the adjacent shade temperature.

ILLUMINATION.

Of the light falling on a leaf, a portion only is available for the chloroplasts since varying amounts, according to the characteristics of the leaf surface, such as the presence of cuticle and of hairs, the thickness of the leaf and so on, will be lost by reflection, absorption or transmission. Of the energy absorbed by the leaf, many have shown that a small proportion only, and this in varying quantity, is used in carbon assimilation. Owing to ignorance of certain factors and the degree of their significance in the sequence of carbon assimilation, it is not possible to give a satisfactory account of the energy relationships of the plant. It may, however, be mentioned that Brown

* Blackman and Smith: "Proc. Roy. Soc." Lond., B. 1911, **83**, 389.
† Blackman and Matthæi: *id.*, 1905, **76**, 402.

and Escombe,* the first to attempt the drawing of an energy
balance sheet of the leaf, concluded that *Polygonum Weyrichii*
used from ·42 to 1·66 per cent of the available radiant energy
for carbon assimilation, figures based partly on observation
and partly on calculation : Puriewitsch,† on the other hand,
found that *Polygonum Sacchalinense* similarly employed from
2·5 to 7·7 per cent of the radiant energy.

Under no natural conditions is the full radiant energy made
use of by plants : the photosynthetic value of the noontide
sunshine at the summer solstice in these latitudes lies, according
to Blackman and Matthæi,‡ between ·04 and ·05 grams of
carbon dioxide per 50 sq. cm. of leaf surface per hour ; the
highest assimilation actually measured by these workers was
·0290 grams.

The general statement that carbon assimilation varies with
the intensity of the illumination is true only when light is the
limiting factor ; other factors, particularly temperature, are
intimately associated in the process in nature.   For this reason
it is impossible to consider the effect of one condition to the
exclusion of the other factors, a fact well demonstrated by
Blackman and Matthæi. §   In one of their experiments, an
abstract of which is given below, the leaves of *Helianthus
tuberosus* were surrounded by an atmosphere containing on the
average 4 per cent carbon dioxide and the light throughout
was diffused and of varying intensity.

Illumination.	Temperature of Leaf ° C.	Real Assimilation in Grams per 50 Sq. Cm. per Hour.
1. Very overcast	17·7	·0062
2. Raining but lighter . . .	18	·0089
3. Raining but lighter still . .	18	·0090
4. Raining, heavy clouds . .	17·7	·0089
5. Raining	30·5	·0163
6. Much brighter	18·2	·0089
7. Sunshine but leaf in shade . .	18·4	·0092 ·

It will be seen from observations 2 to 4 and 6 and 7 that
the assimilation was remarkably uniform under conditions of

* Brown and Escombe : " Proc. Roy. Soc.," Lond., B. 1905, **76**, 29.
† Puriewitsch : " Jahrb. wiss. Bot.," 1914, **53**, 210.
‡ Blackman and Matthæi : " Proc. Roy. Soc.," Lond., B. 1905, **76**, 402.
§ *Ibid.*

constant temperature and varying illumination ; this shows
that temperature was the limiting factor.   The low value in
the first observation was due to the low light intensity.   In
observation 5 in which the illumination was about the same as
in 4, the temperature was raised from $17\cdot7°$ to $30\cdot5°$ C. with
the result that the assimilation was about doubled : but since
at this higher temperature an assimilation of at least $\cdot0289$
grams is possible, it follows that in this instance the illumina-
tion was the limiting factor.

   An important generalization arrived at by F. F. Blackman
and his collaborators is that equal intensities of light incident
on equal areas of leaf produce the same amount of assimilation,
provided that light is the limiting factor and that the tempera-
ture does not involve the so-called time factor ; agreement
within 5 per cent was found to obtain in such diverse instances
as *Helianthus*, *Prunus*, *Bomarea*, *Aponogeton*, *Elodea* and
Fontinalis.   Hence the conclusion is reached that " leaves in
general have the same coefficient of economy in the photo-
synthetic * economy."

   The amount of light required by a leaf is a specific value
for a given temperature ; thus in the examples studied by
Blackman and Matthæi, *Helianthus* and *Prunus* have at low
temperatures similar assimilatory maxima which diverge at
higher temperatures.   At $29\cdot5°$ C. *Helianthus* can assimilate
twice as much carbon dioxide as can *Prunus*, but in so doing
it requires twice the amount of illumination.   The difference
in the two leaves lies in their having different coefficients of
acceleration of their assimilatory activity with increased tem-
perature.

   With regard to the use made by the plant of specific parts
of the spectrum, it has hitherto been generally accepted that
those wave lengths associated with the prominent bands in the
red of the absorption spectrum of chlorophyll were the only
ones concerned with carbon assimilation.   The important
investigations of Ursprung † show that radiant energy of any
wave length to, and including the greater part of, the ultra-
violet is capable of inducing starch formation in green leaves.

---

* The term photosynthetic is used in its narrow sense to refer only to those
radiations specific to this process.

† Ursprung : " Ber. deut. bot. Gesells.," 1917, **35**, 44 ; 1918, **36**, 73, 86, 111,
122.

He even found starch to be formed on exposure to infra-red rays; it is, however, not clear whether this starch was a direct product of carbon assimilation. Further investigation is required on the relationship between carbon assimilation and the specific wave lengths absorbed. Ursprung found in the instance of *Phalaris arundinacea* var. *picta*, which plant was selected since its variegated leaves permit a comparison between the green and non-green portions, that in the red region of the spectrum the maximum absorption obtains between the B and C lines, in the green the absorption is general, whilst in the violet the absorption is greater than between the B and C lines of the red, and beyond this there is a rapid fall. Subsequent work on *Phaseolus* showed a sharp rise in the assimilation curve from the outer limits of the red to a maximum situated near the C line, from which point there is a gradual fall towards the violet. This curve does not correspond, more especially as regards the region of shorter wave lengths, with the curve of absorption which rises from E, in the green region, to the violet. In view of the fact that carbon assimilation takes place in this part of the spectrum, it is obvious that some new conditioning factor is operative; it is suggested that this is found in the action of the violet rays causing the stomates partially to close.

The consideration of the absorption of energy leads to a host of questions regarding the conversion of radiant into other forms of energy, chemical and electrical. And here the realm of hypothesis is reached, for there is no certain knowledge as to the fate of the absorbed energy, the relative and absolute values of specific radiations, and in what form it is dissipated.

With regard to the ultra-violet rays, the more injurious of which are absorbed by the atmosphere, and which in the light of Ursprung's work assume a greater importance, Stoklasa and Zdobnicky * brought about the synthesis of carbohydrate in the absence of chlorophyll by passing light from a quartz mercury lamp through a mica window into a vessel containing a mixture of carbon dioxide and nascent hydrogen.

Formaldehyde was slowly produced and this, in the presence of caustic potash, was polymerized with the formation of a sugar or a mixture of sugars which was optically inactive and not fer-

* Stoklasa and Zdobnicky: "Chem. Zeit.," 1910, 945.

mentable by yeast.   The authors suggested that the chlorophyll
in plants acts as a means of absorbing ultra-voilet rays, a sug-
gestion which has since been found to be true.   Bertholet and
Gaudechon * found that formaldehyde is produced by the action
of ultra-violet rays on carbon dioxide in the presence of a re-
ducing agent, and, with regard to the reverse process, that
carbohydrates are decomposed by sunlight and by ultra-violet
light from a mercury lamp.   The products of decomposition
are carbon monoxide, carbon dioxide, methane and hydrogen ;
aldehydic sugars differ from ketonic sugars both in the readiness
with which they are decomposed and in the composition of the
gaseous mixtures produced.

Usher and Priestley † found that ultra-violet light can bring
about the decomposition of aqueous carbon dioxide without
the intervention of an optical or chemical sensitizer, a result
contrary to Stoklasa and Zdobnicky who found that formaldehyde
was not produced by the action of ultra-violet light on carbon
dioxide and water.   In view of these contrary results, Spoehr ‡
tried the effect of ultra-violet radiations on carbonic acid and its
salts ; in no experiment was a sufficiency of formaldehyde pro-
duced to give a positive reaction with the reagents employed.
In all experiments Spoehr found that formic acid was the only
reduction product.   Attempts to reduce formic acid to formalde-
hyde by sun or by ultra-violet light failed, but after ten to fifty
hours exposure there remained on evaporation a non-volatile
yellow syrup, of a composition not yet determined, which re-
duced Fehling solution.

The subject has received renewed attention from Baly,
Heilbron, and Barker § who find that an aqueous solution of
carbon dioxide yields formaldehyde when exposed to light of
very short wave length, 200 $\mu\mu$.   Under the influence of light
of wave length 290 $\mu\mu$, however, formaldehyde in water is
polymerized to reducing sugars, but if substances, sodium
phenoxide for example, which absorb this wave length and
which are ineffective in the chemical actions involved, are

* Bertholet and Gaudechon : " Compt. rend.," 1910, **150,** 1690, **151,** 395 ;
1912, **155,** 401, 831.
† Usher and Priestley : " Proc. Roy. Soc.," Lond., B. 1911, **84,** 101.
‡ Spoehr : " Plant World," 1916, **19,** 1.
§ Baly, Heilbron, and Barker : " Journ. Chem. Soc.," Lond., 1921, **119,**
1025.

present, the amount of formaldehyde is increased in an aqueous solution of carbon dioxide, since the action of such substances is to protect the formaldehyde from polymerization.   These are test tube observations : the plant can flourish under conditions which entirely withhold radiant energy of these short wave lengths.   In such cases a photocatalyst is required and Baly and his collaborators find that in ordinary visible light the photosynthesis of formaldehyde and its polymerization to carbohydrate can be achieved in two separate stages : in the first stage formaldehyde is produced from carbon dioxide and water in the presence of coloured basic substances such as methyl orange ; in the second stage formaldehyde is polymerized to a reducing sugar, without the aid of a photocatalyst, when exposed to the rays from a quartz mercury lamp.

The authors point out that if a photocatalyst capable of bringing about both changes in the same vessel in the laboratory were known, then the separate existence of formaldehyde would not be demonstrable since the formaldehyde produced from carbon dioxide and water would at once be polymerized into carbohydrate.   Such a photocatalyst has yet to be found, and if chlorophyll be one such, then the small amount of formaldehyde in carbon-assimilating leaves is to be expected. This investigation is still in progress : as yet Baly and his fellow-workers have not found the desired photocatalyst and so far have not ascertained to what degree chlorophyll meets their requirements in this respect ; their results are not in accord with those of Osterhout * nor do they examine the contention of Spoehr, outlined above, regarding formic acid.

Chemical change may be brought about by electrical energy ; indeed, in connection with plants, the effect of electrical currents on vegetable growth is a not unimportant branch of applied botany.

Royer † brought about the electrolytic reduction of carbon dioxide, and by similar means Coehn,‡ in 1904, produced formic acid from this same compound.   Brodie § found that by means of a silent discharge formaldehyde, together with

* See Vol. I., p. 61.
† Royer : " Compt. rend.," 1870, **70, 731.**
‡ Coehn : " Ber. deut. chem. Gesells.," 1904, **34,** 2836, 3593.
§ Brodie : " Proc. Roy. Soc.," Lond., 1874, **22,** 171.

marsh gas, was produced from a mixture of hydrogen and carbon dioxide ; and Löb, * in 1906, found that formaldehyde may be produced by the action of a silent discharge of electricity through a solution of carbon dioxide in water. Fenton † also has pointed out that the synthetic action of light and of the silent electrical discharge are practically identical. Thus there is evidence which suggests that electric energy may play a part in the earlier processes of photosynthesis ; a suggestion which is supported by the fact that, according to Polacci, ‡ the formation of carbohydrates is promoted in leaves by electrical energy, provided it be not too intense, especially when a continuous current is made to pass directly into the tissues.

As a result of a number of experiments, Gibson § comes to the conclusion that the light rays which are absorbed by the chlorophyll are transformed into electrical energy, and it is this transformed energy which brings about the decomposition, of carbonic acid to formaldehyde and oxygen. This opinion is based on evidence the complete details of which apparently have not been published.

With regard to other forms of energy, attention may be drawn to the work of Kernbaum,‖ who found that water exposed to the influence of β rays and of ultra-violet rays led to the production of hydrogen and hydrogen peroxide. Usher and Priestley also found that an aqueous solution of carbon dioxide could be decomposed by the a and β rays from radium emanation. The action of ·0001 c.c. of radium emanation on 200 c.c. of water saturated with carbon dioxide resulted in four weeks in the production of hydrogen peroxide and formaldehyde. Most of the latter was in a polymerized state, but the solution contained no sugar.

Stoklasa ¶ considers that the essentiality of potassium, which is feebly radioactive, to the well-being of green plants is in part due to this property which is associated in the

* Löb : " Zeit. Electrochem.," 1906, 12, 282.
† Fenton : " Journ. Chem. Soc.," Lond., 1907, 91, 687.
‡ Polacci : " Atti. Inst. Bot.," Pavia, 1905, II., 11, 7.
§ Gibson : " Ann. Bot.," 1908, 22, 117.
‖ Kernbaum : " Compt. rend.," 1909, 148, 755, 149, 273.
¶ Stoklasa : " Biochem. Zeitsch.," 1920, 108, 109.

transformation of energy in the photosynthetic phase of carbon assimilation.

INTERNAL FACTORS.

CHLOROPHYLL.*—Plant physiologists for long have recognized that the intensity of carbon assimilation must be dependent on the chlorophyll and its amount ; it is, however, but recently that the problems involved have been critically examined. Irving,† who used the leaf's carbon dioxide of respiration in her experiments, found by gasometric methods that etiolated leaves, either when they are orange-yellow or when they have attained a considerable degree of greenness, do not possess any appreciable power of synthesizing carbon dioxide. If there be any photosynthetic activity, it cannot be greater than one-tenth part of respiration nor come within 1 per cent of the activity subsequently developed. Carbon assimilation begins when the leaves are fully green and develops very quickly ; wherefore it follows that the first origin of this function is not correlative to the amount of chlorophyll produced, or, in other words, that the amount of chlorophyll is not a conditioning factor in the early stages of carbon assimilation.

Willstätter and Stoll ‡ were the first to make quantitative estimations of the amount of chlorophyll in leaves, by the methods already outlined.§ Also they measured the amount of carbon assimilation of the leaves of different plants and of the same plant in different conditions—normal, etiolated, autumnal, and so on—and thus arrived at the assimilation number which is the ratio between the amount of carbon dioxide assimilated per hour and the chlorophyll content both expressed in milligrams. A selection of the values obtained are tabulated below.

Willstätter and Stoll, whose experimental methods were similar to Irving's, with the chief exception that they used a 5 per cent concentration of carbon dioxide, found that

---

* A general account of chlorophyll, its chemistry and constitution, will be found in Vol. I.

† Irving : " Ann. Bot.," 1910, **24**, 805.

‡ Willstätter and Stoll : " Ber. deut. chem. Gesells.," 1915, **48**, 1540.

§ See Vol. I., Section on Pigments.

leaves with but a small portion of their full chlorophyll content developed can assimilate to a measurable degree.

WILLSTÄTTER AND STOLL'S ASSIMILATION NUMBERS.

Plant.	Kind of Leaf.	Chlorophyll Content in Mgs.	CO_2 Assimilated per Hour Mgs.	Assimilation Number.
Primula	Normal	11·4	105	9·1
Rubus	Normal	16·2	94	5·8
Tilia	Young	5·2	74	14·2
	Older	22·5	148	6·6
Populus	Dark green autumn leaves	15·2	152	10·0
	Yellow-green autumn leaves	3·9	31	7·9
Elm	Yellow variety	0·95	75	7·9
	Green variety	13·0	89	6·9

This conclusion is contrary to that of Irving, a difference probably due to the fact that Irving used young leaves whilst Willstätter and Stoll employed older and sometimes much older material. This explanation is due to Briggs,* in whose memoir a critical examination of the work of the above-mentioned authors will be found; this author demonstrates that the age of a leaf and the lapse of time from the greening to the measurement of photosynthetic activity are all important. If a leaf is cut from a seedling in the dark at an early stage in its development and partly greened by exposure to light, its photosynthetic activity will be zero or very small; if, on the other hand, the same procedure is repeated with a similar leaf from the same plant after an interval of a few days, the photosynthetic activity will be strongly marked. Briggs confirms Irving's main conclusions: a young green leaf may show no or very little carbon assimilation and the power of photosynthesis lags behind the development of chlorophyll. This power increases with age whether the leaf be in the dark or in the light even though there be no concurrent increase in the chlorophyll content.

THE UNKNOWN FACTOR.— The fact that the temperature relations of carbon assimilation are those of a chemical rather than a photochemical reaction indicates the presence of an internal factor, independent of the chlorophyll and associated rather with the protoplasm, which controls the rate of carbon

* Briggs : " Proc. Roy. Soc.," Lond., B. 1920, 91, 249.

assimilation. Irving concludes that this factor controls the beginning of the process since it is not developed so quickly as the chlorophyll, wherefore the rest of the mechanism must await its appearance.

The assimilation numbers arrived at by Willstätter and Stoll * are inconstant, which is indicative of there being some other operating factor : if they were constants, strong evidence that chlorophyll was the all-important conditioning factor would be provided. According to Willstätter and Stoll † it is an enzyme which thus limits carbon assimilation. They find that in leaves rich in chlorophyll, increased illumination has but little effect upon assimilation nor is it diminished if the illumination is decreased to one quarter. This indicates that the chlorophyll is present in excess compared with the assimilatory enzyme. The increase in carbon assimilation following an increase in temperature they consider to be due to the stimulation of the enzymatic process (cf. p. 48).

In leaves containing little chlorophyll and in yellow varieties, the conditions are reversed ; the enzyme here being in excess, increased temperature has little effect in stimulating assimilation. On the other hand, increased illumination has a very marked effect.

The remarkable phenomena accompanying autumnal changes in leaves are due to the fact that either the chlorophyll suffers more than the enzyme, resulting in increase of assimilation number, or conversely the enzyme suffers most, in which case the assimilation number falls.

The failure to bring about carbon assimilation by means of chlorophyll isolated from the leaf may be attributed, according to the authors, to the absence of the enzyme. Also they observed that carbon assimilation continued in leaves from which the lower epidermis had been removed whilst a slight pressure applied to the leaves brought the process to a standstill. This is explained on the assumption that the enzyme is situated at the surface of contact between the chloroplast and the plasma.

This belief in the existence of a controlling enzyme also is shared by Osterhout and Haas ‡ who experimented with

* Willstätter and Stoll : " Ber. deut. chem. Gesells.," 1915, **48**, 1540.
† *Id.*, p. 1552.
‡ Osterhout and Haas : " Proc. Nat. Acad. Sci.," 1918, **4**, 85.

various plants, both fresh water and marine, grown in a culture solution containing gaseous carbon dioxide. The removal of carbon dioxide was followed by the method of determination of the hydrogen ion concentration in the culture medium. It was found that photosynthesis began immediately on exposure to light; at first the removal of carbon dioxide was rapid, then more slowly until a maximum was reached when the rate remained constant. These authors consider that under the influence of light some catalyst is developed which facilitates the photosynthetic process. Finally Briggs concludes that the activity of the photochemical phase of the carbon assimilation mechanism in a seedling, as compared with that of more mature leaves, is in some way limited : the photochemical phase depends for its intensity not only on the chlorophyll but also upon some other factor which increases with age during the early stages of leaf development.

There is thus strong evidence of the existence of a controlling factor essential in the earlier phases of carbon assimilation : whether it be enzymatic or protoplasmic, it is impossible to say.

The Products of Carbon Assimilation.—Much investigation is necessary before the precise significance of this factor can be determined : of the products of carbon assimilation, carbohydrate and oxygen are the most conspicuous; others there are, but it is unprofitable, if not impossible, to consider them on this immediate occasion since accurate knowledge of their part in carbon assimilation does not exist.

It is well known that according to the law of mass action, chemical action diminishes as the products of the activity accumulate ; the initial rate can only be maintained provided the initial proportions of the reacting substances are preserved and the products of the reaction removed. In the leaf a further complication arises since sugar is an osmotic substance and its undue accumulation may lead to a physical disturbance in the synthesizing machine such, for example, as the closure of the stomates. This may be averted by the conversion of the sugar into starch, a marked and well known phenomenon in many plants, which temporary reserve is translocated at nightfall after hydrolysis. Translocation is most apparent

in the dark, but it takes place also by day and it is only in sugar leaves that the undue concentration of soluble carbohydrates, owing to hindrances to rapid translocation, or to a high assimilatory efficiency, is likely to prove an important factor in carbon assimilation.   In this respect the observations of Broock * are of considerable interest and value.   He found that the leaves of the sugar beet on bright sunny days showed a rapid increase in their dry weight up to mid-day, at which hour there was a sudden decrease: this loss in weight was more or less uniform until about midnight.   The sudden drop in the carbon assimilation at noon indicates that the process was brought to a standstill owing, in all probability, to the closure of the stomates resulting from the accumulation of materials which were produced at a greater rate than that at which they could be translocated.

With regard to the oxygen evolved, since carbon assimilation by green leaves is unaffected by various concentrations of oxygen in artificial atmospheres, and since the oxygen pressure of the internal atmosphere of plants is a very variable quantity, it would not appear that the amount of oxygen is a limiting factor of any great significance.

THE ORGANIC PRODUCTS OF CARBON ASSIMILATION.

The ultimate products of carbon assimilation are carbohydrates : Brown and Morris,† in their classical work on the chemistry and physiology of foliage leaves, identified sucrose, dextrose, levulose, and maltose in the leaves of *Tropæolum*, the sucrose being in greatest abundance.   These results were generally accepted, reinvestigations of the subject being of recent date.   Parkin ‡ in his investigation of the sugars of the leaf of *Galanthus nivalis*, selected for its convenience as a sugar leaf, found that a considerable quantity of sugars, 20 to 30 per cent of the dry weight, occurred in the active leaves ; sucrose, dextrose, and levulose were recognized but maltose was never found, a result which was to be expected, since starch is not

* Broock: " Über tägliche und stündliche Assimilation einiger Kulturpflanzen. Halle: 1892. See Thoday : " Proc. Roy. Soc.," Lond. B., 1910, **82**, 443.

† Brown and Morris : " Journ. Chem. Soc.," Lond., 1893, **63**, 604.

‡ Parkin ; " Biochem. Journ.," 1912, **6**, 1.

formed in the leaves in any appreciable quantity.   The amount
of sugar increases towards the base of a leaf and concurrently
the ratio of the sucrose to the hexoses diminishes.   The sucrose
increases rapidly in amount after sunrise, reaches a maximum
and diminishes during the night, the percentage amounts of
the hexoses of the leaf, however, remain fairly constant.   As
the season advances, the proportion of sucrose to the hexoses
decreases, the latter being formed at the expense of the former.
Parkin confirms Brown and Morris in the observation that the
levulose is, as a rule, more abundant than the dextrose and
supports the contention that sucrose is the first recognizable
sugar in carbon assimilation, the hexoses being formed from
it by inversion.   The reason for levulose being more abundant
than dextrose is that the latter is more used by the leaf in its
respiratory processes.

Davis, Daish, and Sawyer,[*] in the instances of the potato
and the mangold, agree that sucrose is a primary sugar of
carbon assimilation and from it the hexoses arise.   The cane
sugar and hexose are at their maximum at mid-day and the
amounts increase with the season.   Unlike Brown and Morris
they found pentose to be present whilst maltose was consist-
ently absent, both by day and by night, from the leaves men-
tioned.   In view of the presence of starch in the potato leaf,
it is surprising that the presence of maltose was not estab-
lished: there is good reason for supposing that maltase is
commonly present in plants in which starch occurs and is
digested: this enzyme is rather indiffusible, is destroyed by
alcohol and chloroform under ordinary conditions, and is
very susceptible to heat, a temperature of $50°$ C. having a
marked adverse effect upon its activity, whilst at $55°$ C. it may
be destroyed.   It is these properties of maltase which account
for the varying statements regarding the occurrence of mal-
tose in green leaves : if the procedure of experiment is such
as to destroy the maltase, maltose will be found; if, on the
other hand, the enzyme is not destroyed, the maltose is con-
verted into hexose sugars.[†]

Gast [‡] examined the carbohydrate content of the leaves of

[*] Davis, Daish, and Sawyer : " Journ. Agric. Sci.," 1916, **7**, 225.

[†] Davis : " Biochem. Journ.," 1916, **10**, 31, 49, 56.

[‡] Gast : " Zeitsch. Physiol. Chem.," 1917, **99**, 1.

Tropæolum, Vitis, Musa and other plants collected in the early afternoon and early morning: he found that sucrose always was more abundant than maltose and the other sugars identified; he is in accordance with Brown and Morris regarding the absence of pentose. Kylin [*] found that in general terms the amount of glucose varies inversely as the amount of starch. Sucrose is generally the most abundant sugar, especially in sugar leaves, but *Gentiana brevidens*, a sugar leaf, does not contain any sucrose, whilst *Tilia europæa*, a starch leaf, contains much sucrose.

In conclusion it may be said that leaves generally contain sucrose, maltose, dextrose, and levulose, whilst the occurrence of pentose is uncertain.

With regard to starch, its occurrence and relationships to sugar have already been considered, [†] and since agreement regarding the sequence of events in carbon assimilation which culminate in the formation of sugar has not been reached, it is not desirable here to discuss which is the primary sugar of the synthetic process.

Fats sometimes have been described as direct products of carbon assimilation, more especially in plants, such as *Vaucheria*, which have an abundance of fat-like substance in their green tissues. The evidence for the contention is anything but satisfactory, especially in view of the transmutation of carbohydrate into fat in various plants and under the influence of certain external conditions such as low temperature. [‡] The problem has been resuscitated by Meyer § who describes the presence of a colourless fluid of unknown composition and having the physical properties of an oil which occurs in the chloroplasts of *Tropæolum*. This substance, which is not a fat, since it gives on distillation hexylenealdehyde, increases with assimilation, and is considered by Meyer to be a definite product of carbon assimilation.

HYPOTHESES CONCERNING THE SYNTHESIS OF CARBO-HYDRATE BY THE GREEN PLANT.

With the problems associated with the march of events from the initial carbon dioxide and water to the final carbo-

---

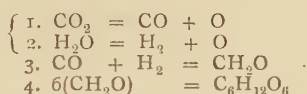[*] Kylin: *id.*, 1918, **101**, 77.　　　　[†] Vol. I.　　　　[‡] See Vol. I.
§ Meyer: "Ber. deut. bot. Gesells.," 1917, **35**, 586; 1918, **36**, 235, 674.

hydrate, the realms of theory are entered.   The hypotheses
are many and have this in common—none entirely satisfy the
plant physiologist ; for which reason it is proposed to consider
but two in any detail on the present occasion, not that those
discarded are barren of ideas—indeed, some contain valuable
suggestions—but that their examination, more especially when
unsupported by experimental evidence, would tend rather to
obscure than to clarify the problem.

In 1870 Baeyer put forward the hypothesis that the carbon
dioxide is split up by the plant into carbon monoxide and
oxygen, and that the water is concurrently resolved into its
constituent elements.   The carbon monoxide and hydrogen
thus produced then combine to produce formaldehyde, which
undergoes polymerization, and so forms a hexose.

These changes may be represented in the following equa-
tions :—

$$\begin{cases} 1. \ CO_2 = CO + O \\ 2. \ H_2O = H_2 + O \end{cases}$$
$$3. \ CO + H_2 = CH_2O$$
$$4. \ 6(CH_2O) = C_6H_{12}O_6$$

Thus, according to the theory, there are two distinct actions,
the first leading to the formation of formaldehyde, and the
second to the production of sugar.

Considering the first part of Baeyer's theory, it is seen that
both carbon monoxide and hydrogen are supposed to be pro-
duced, but carbon monoxide has been found but once in a free
state in the living plant (p. 73), and it is a substance which
does not lend itself at all readily to constructive metabolism,
the evidence as to whether plants are able to make use of it
for synthetic purposes being contradictory.   Bottomley and
Jackson [*] state that if the carbon dioxide normally present
in the atmosphere be replaced by about twenty times as much
carbon monoxide—the increase in the amount of the latter
being necessary on account of its lesser solubility in water as
compared with carbon dioxide—plants of *Tropæolum* formed
starch and flourished.   Preliminary analyses also showed that,
in the case of seeds germinated in an atmosphere in which the
carbon dioxide had been replaced by carbon monoxide, there
was in the seedlings an increase in organic carbon.   Further,

[*] Bottomley and Jackson : " Proc. Roy. Soc.," Lond. B., 1903, **72**, 130.

they found that a negative pressure obtained in the vessels containing the plants assimilating carbon monoxide. This was to be expected if the hypothesis be accepted, for if the carbon monoxide be used up in photosynthesis, then the amount of oxygen set free would be half that evolved in normal photolysis.   On the other hand, Kraschénnikoff [*] has come to the conclusion, based on a number of experiments, that green plants cannot make use of carbon monoxide; he points out, however, that his evidence does not prove that carbon monoxide is not formed in the early stages of photosynthesis.   It may also be remarked that according to the investigations of Sulander,[†] carbon monoxide acts as an anæsthetic, but is much weaker in its action than chloroform.   He found that 0·5 per cent of this gas was sufficient to inhibit the growth of seedlings of the lupin and the germination of the spores of many Fungi.   It does not therefore follow that carbon monoxide is not formed in plants.   As is well known, carbon dioxide is itself an anæsthetic if present in a sufficient amount, and possibly it is more potent in this respect than is carbon monoxide, for Sulander found that in many cases the streaming movements of protoplasm were not affected even after several hours' exposure to the last-named gas.

A modification of Baeyer's theory thus appears to be necessary.   Erlenmeyer, long before the experimental work on carbon monoxide just referred to was done, suggested that the carbonic acid in the cells undergoes a reduction which leads to the formation of formic acid and oxygen, and that the formic acid is further reduced to formaldehyde and oxygen :—

$$1.\ H_2CO_3 = CH_2O_2 + O$$
$$2.\ CH_2O_2 = CH_2O + O$$

or else that the action is continuous and that the carbon dioxide and water may directly give rise to formaldehyde and oxygen :—

$$CO_2 + H_2O = CH_2O + O_2$$

According to these views either formaldehyde or formic acid must be produced.

* Kraschénnikoff : " Rev. gén. Bot.," 1909, **21**, 177.
† Suland.r : " Beih. bot. Centrbl.," 1909, **24**, I., 357.

These substances, as is well known, are poisonous, so that, if formed, they must be polymerized before they have time to injure the protoplasm, and experiments have shown that, under certain conditions, formaldehyde may be made use of by the plant.   Thus Bokorny * showed that *Spirogyra* in the absence of carbon dioxide can make starch when supplied with a compound of formaldehyde and sodium hydrogen sulphite ; also Treboux † and Bouilhac have stated that *Elodea, Sinapis*, and certain Algæ can form starch in the dark when supplied with dilute (·0005 per cent) solutions of formaldehyde.

Grafe ‡ found that green seedlings grown in the light, in an atmosphere free from carbon dioxide and containing not more than 1·3 per cent of formaldehyde vapour showed a greater increase in growth and in dry weight as compared with the controls.   These results were corroborated by Baker § who concluded from most carefully controlled experiments that formaldehyde could be assimilated to a certain extent in the light, but not in the dark, in an atmosphere void of carbon dioxide.

With regard to the presence of formaldehyde in green leaves there is much positive evidence ‖ and mention has already been made of the formation of formaldehyde and of formic acid from carbon dioxide and water under the influence of different forms of energy.

Willstätter and Stoll ¶ argue that the " assimilatory ratio," CO_2/O_2, should provide evidence regarding the possible intermediate products ; they point out that oxalic, formic and glyoxylic acids and formaldehyde are possible intermediate products between the carbon dioxide and carbohydrate.   The assimilatory ratios of these substances are respectively, 4, 2, 1·33 and 1.   Under the conditions of their experiments, they found the assimilatory ratio to be 1 and it remained constant, even in succulents, under varying conditions in the amounts of carbon dioxide and oxygen and with the temperature ranging

* Bokorny : " Biol. Zentrbl.," 1897, **17**, 1, 1916, **36**, 385 ; " Ber. deut. chem. Gesells.," 1891, **24**, 103.
† Treboux : " Flora," 1903, **92**, 49.
‡ Grafe : " Ber. deut. bot. Gesells.," 1911, **29**, 19.
§ Baker : " Ann. Bot.," 1913, **27**, 411.
‖ See Vol. I., Section on Aldehydes.
¶ Willstätter and Stoll : " Ber. deut. chem. Gesells.," 1917, **50**, 1777.

from 10° C. to 35° C.   Wherefore, it is concluded that the carbon dioxide is reduced to formaldehyde, this being the only hydrate having a single carbon atom.

Other authors consider that formic acid is the more likely intermediate product of early origin.   Erlenmeyer was the first to make the suggestion, but it is only of recent years that renewed attention has been given to the possibility.   The work of Spoehr has already been mentioned : he demonstrated that carbon dioxide and water are easily reduced to formic acid by means of radiant energy ; from formic acid a sugar-like substance is formed, under conditions such as obtain in an active leaf, which the plant can utilize as a food stuff.   He points out that in Grafe's and Baker's work the plants were in reality supplied with formic acid, since formaldehyde is oxidized in the light, and he himself found that plants increased in dry weight in an atmosphere of formic acid.   Wislicenus [*] also supports the formic acid hypothesis.

Laboratory experiments on the polymerization of formaldehyde to sugars not infrequently are quoted in support of the formaldehyde hypothesis.

The first successful attempt to bring about such a polymerization was made by Butlerow in 1861, who, by the catalytic action of lime water, at the ordinary temperatures, on trioxymethylene (itself a polymer of formaldehyde), obtained a syrup with a somewhat bitter taste, which he called methylenitan. Subsequently Loew undertook an investigation of the action of milk of lime on formaldehyde, and devised the following experiment.   A 4 per cent solution of formaldehyde is mixed with an excess of milk of lime and repeatedly shaken for about half an hour ; after filtering, the mixture is set aside for some days until the pungent smell of formaldehyde has disappeared. The solution, which will now reduce Fehling's solution, yields a colourless syrup with a very sweet taste.[†]   This substance, which is known as crude formose, was examined by Emil Fischer, who found it to consist of a mixture of various

---

[*] Wislicenus : " Ber. deut. chem. Gesells.," 1918, **51**, 942.

[†] More recently Ewart (" Proc. Roy. Soc.," Victoria, 1919, **31**, 328) has worked out the best conditions for the polymerization of formaldehyde to sugar, and finds that the most rapid reaction is produced by sodium hydroxide in the presence of a neutral calcium salt.

hexoses and succeeded in isolating from it a small quantity of a sugar—acrose.   Acrose can also be obtained by the action of dilute caustic soda on glycerose, a substance obtained by the oxidation of glycerol.   From the acrose thus formed Fischer was able by an elaborate series of reactions to prepare ordinary fructose or levulose.   In respect to the action of weak alkalis, Spoehr found that sunlight had no action on a 3 per cent solution of formaldhyde in the presence of such salts as calcium carbonate, potassium carbonate and potassium nitrate in decinormal concentration, no trace of sugar being found after an isolation period of four months.

With regard to the formation of the higher carbohydrates from fructose, practically nothing is known.   Whilst the synthesis of levulose from formaldehyde *in vitro*, and the conversion of levulose into glucose are established facts, no success has as yet attended attempts to synthesize sucrose from its two hexose sugars, dextrose and levulose.   In this connexion, however, it may be mentioned that the synthesis of disaccharides from monosaccharides has been achieved only in a very few cases.   Fisher and Armstrong * were able to synthesize a disaccharide—isolactose—by the action of an enzyme, Kefir lactase, on a mixture of glucose and galactose ; the same authors also synthesized melibiose.   Similarly isomaltose has been obtained by Croft-Hill † by the action of maltase on glucose.

It is obvious from this survey that the hypothesis of Baeyer has influenced to a remarkable degree investigations on the synthetic aspects of carbon assimilation ; this to a large extent is psychological, for the hypothesis is attractive in that it is plausible and lends itself readily to test tube investigation. The evidence readily centres around distinct nuclei—the identification in the plant of the critical initial intermediate products ; the effect of presenting the plant with some of these initial substances in place of natural raw material ; and the elaboration of the final from the supposed initial products in the laboratory —and may be briefly considered under these heads :—

Carbon Monoxide.—This gas, if produced, must be fixed

* Fisher and Armstrong : " Ber. deut. chem. Gesells.," 1902, **35**, 3144.
† Croft-Hill : " Journ. Chem. Soc.," Lond., 1898, **73**, 634 ; see also Emmerling : " Ber. deut. chem. Gesells," 1901, **34**, 600, 2206.

with extreme rapidity, for there is no evidence of its occurrence as such in assimilating tissues, remarkable for a compound so inert. The balance of evidence is against its use by the plant when substituted for carbon dioxide.

Formaldehyde.—There is no doubt that formaldehyde can be obtained from green leaves, there being many compounds which yield it more or less readily on decomposition. The production of formaldehyde in artificial chlorophyll systems was once considered strongly to support Baeyer's hypothesis,[*] but the work of Jörgensen and Kidd, who were the first to use pure chlorophyll in this connexion, seriously discounts the significance of the reactions since the formaldehyde has its origin in the oxidation of the chlorophyll ; it is not produced in such systems if oxygen be absent. Much of the evidence relating to the production of formaldehyde from carbon dioxide and water under the influence of radiant energy is contradictory. The work of Baly and Heilbron, however, has placed this aspect of the subject on a much firmer basis.

The facts relating to the production of formaldehyde in the presence of catalysts, although of interest in themselves and important in other connexions, provide no proof that such reactions obtain in the normal plant.

The contention of Willstätter and Stoll based on the assimilatory ratio is ingenious, but, since the formaldehyde was not actually demonstrated, the evidence is not convincing.

Formic Acid.—As compared with formaldehyde the formic acid hypothesis has received much less attention : Spoehr's judgment appears to be just ; there is more in its favour than for formaldehyde.

Feeding Experiments.—That plants under abnormal conditions, deprived of their natural raw food materials, may make use of other substances in the elaboration of their food shows that the plant is a transcendental chemist and in virtue of its powers can to a certain degree make use of the substitutes. The facts do not prove that these substitutes, formaldehyde and formic acid, are normal intermediate products in the natural process. If, on the other hand, it is held that

---

[*] See, for example, Usher and Priestley : " Proc. Roy. Soc.," Lond., B., 1906, **77**, 369 ; 1906, **78**, 318 ; 1911, **84**, 101.

this kind of evidence is of direct value then the weight of evidence is on the side of formic acid.

The Production of Sugar from Formaldehyde.—The evidence for the condensation of formaldehyde to hexose sugars in the laboratory under the influence of various catalysts is convincing : but valuable though such evidence be, especially in providing points d'appui, it does not follow that such a sequence obtains in the plant.   The formaldehyde hypothesis requires a hexose as the initial sugar : the general opinion of those who have concerned themselves with the final products of carbon assimilation is that sucrose, not a hexose, is the first recognizable sugar.

For these reasons, Baeyer's hypothesis, a theory which has lived for fifty years, cannot be considered to be substantiated.

Willstätter and Stoll's views regarding the mechanism of carbon assimilation may now be summarized.

They agree with Baeyer that formaldehyde is the intermediate link between the carbon dioxide supplied to the plant and the carbohydrate synthesized with the help of chlorophyll. It has already been pointed out that their chief argument in support of this view is based upon the constant value, 1, obtained for the so-called assimilatory ratio; this ratio is obtained by dividing the volume of carbon dioxide supplied to the plant by the volume of oxygen evolved ; according to theory, the complete reduction of carbon dioxide, i.e. the removal of both oxygen atoms, might proceed through successive stages of oxalic, formic and glycollic acids down to the formaldehyde stage, but in that case the assimilatory ratio would be respectively 4, 2, or 1·33 for each of the acids mentioned, whereas formaldehyde alone requires the value 1 which is the figure actually obtained in a number of experiments under most varied conditions.   This argument is not based upon the actual experimental demonstration of formaldehyde isolated from assimilating leaves, and, with regard to this particular question, these authors have revised many of the statements made by previous workers.   It will be remembered that repeated attempts have been made to demonstrate the formation of formaldehyde by the action of chlorophyll upon carbon dioxide outside the plant.   The earlier work of Usher and Priestley, demonstrating the formation of this substance in

films of chlorophyll exposed to an atmosphere of carbon dioxide in sunlight, was shown by Wager * and Warner † to be faulty, inasmuch, as Wager showed, that no formaldehyde was produced if oxygen was excluded, whilst Warner showed that carbon dioxide was unnecessary and took no part in the production of the formaldehyde.   Warner concluded that the formaldehyde was in fact an oxidation product of the chlorophyll since oxygen was actually absorbed in the process.   This view has, however, since been superseded by the experiments of Willstätter and Stoll who showed that no formaldehyde at all was formed if pure chlorophyll in colloidal solution was employed, the colloidal solution being considered to approximate most closely to the condition of the chlorophyll in the chloroplast. The formaldehyde described by the earlier workers is attributed to the oxidation of impurities accompanying the samples of chlorophyll used by them.

The failure to obtain any trace of formaldehyde from pure chlorophyll *in vitro* is attributed by Willstätter and Stoll to the absence of the essential enzyme which these authors postulate in the green leaf, and this brings us to the consideration of the mechanism of the action of chlorophyll upon carbon dioxide as visualized by these authors.

Experiments *in vitro* have shown that carbon dioxide can form with chlorophyll (I.) in colloidal solution an additive compound of the type of a bicarbonate (II.) as expressed by the equation

$$R \begin{cases} >N \\ Mg \cdot H_2O \cdot CO_2 \\ >N \end{cases} = R \begin{cases} >N \\ Mg - O.C \stackrel{O}{\underset{OH}{}} \\ >NH \end{cases}$$
$$ \text{I} \text{II}$$
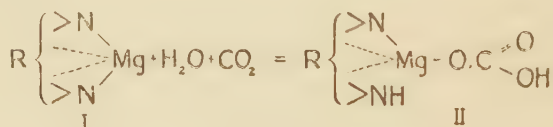
This compound (II.) cannot be imagined to be capable of parting with two atoms of oxygen with regeneration of chlorophyll, so that some intramolecular rearrangement must first take place, and this, according to Willstätter and Stoll, involves the absorption of energy which is supplied by the sunlight.

* Wager: " Proc. Roy. Soc.," Lond. B., 1914, 87, 386.
† Warner: *id.* 378.

This change to a formaldehyde peroxide compound (III.) may be illustrated as follows:—
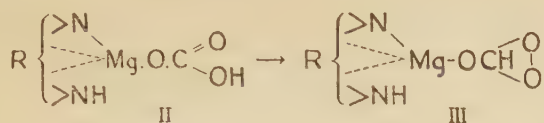
It is true that experiments *in vitro* have entirely failed to demonstrate the formation of a peroxide compound by means of horseradish peroxidase, but this is considered to demonstrate the essential difference between test tube experiments and the activity of the living cell.   There is no doubt that chlorophyll in the chloroplast is protected from photo-oxidation or decomposition by carbon dioxide in a way that chlorophyll in colloidal solution *in vitro* is not, since the chloroplast will tolerate concentrations of carbon dioxide which decompose chlorophyll in colloidal solution to the magnesium free compound phaeophytin (V.) with precipitation of magnesium carbonate as illustrated by the equation

$$C_{55}H_{72}O_5N_4Mg + H_2O + CO_2 = \underset{V.}{C_{55}H_{74}O_5N_4} + MgCO_3$$
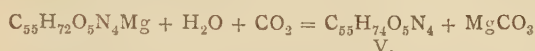
Such a peroxidic compound (III.) as is postulated above should be fairly easily capable of losing oxygen either in one or in two stages with regeneration of unaltered chlorophyll and formation of formaldehyde (IV.)
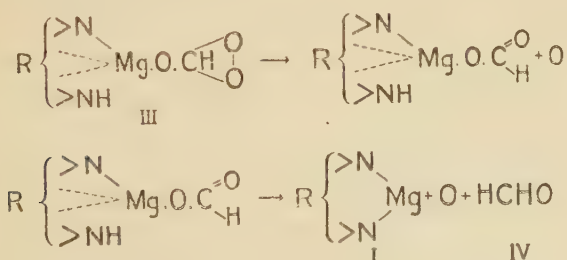
Within the living cell the decomposition of the peroxide formaldehyde compound (III.) is assumed to be brought about by an enzyme, the existence of which enzyme is supported by the following experimental evidence.

The assimilation number is the ratio of the number of milligrams of carbon dioxide assimilated to the number of milligrams of chlorophyll in the assimilating leaf surface, i.e. $\dfrac{CO_2}{\text{chlorophyll}}$.

In leaves in which the chlorophyll content is small, the ratio will be higher than in those in which it is large. In such leaves the proportion of enzyme will be comparatively large, with the result that conditions which favour enzyme activity, such as temperature, will have but little effect upon the assimilation number; increased illumination, however, should assist the chlorophyll which is comparatively deficient; on the other hand, in cases where the chlorophyll is in excess, only those conditions which can assist enzyme activity will have any effect upon the assimilation number. Experimental results entirely bear out these theoretical considerations and so support their correctness.

In this connection further reference to the views of Baly and Heilbron upon the formation of carbohydrates in the plant may be made. The authors point out that to effect the change represented by the equation—

$$CO_2 + H_2O = HCHO + O_2$$

energy must be supplied since the energy contents of the products are greater than those of the reactants. Since an aqueous solution of carbon dioxide is unable to absorb visible light but will absorb ultra-violet light of short wave length, it is the latter kind of radiation which will normally be required to effect the change. Since, however, ordinary sunlight contains but little of such short wave-length light, it is assumed that the function of the chlorophyll is to absorb the visible light and to radiate infra-red energy of a frequency identical with that of the carbon dioxide, which can then absorb it; thus the photo-synthetic process can proceed in the absence of the ultra-violet light normally required for the process. In this capacity the chlorophyll is said to act as a photocatalyst. The authors have shown experimentally that while formaldehyde is synthesized from carbon dioxide and water by short wave-length ultra-violet light, it is polymerized to carbohydrate by ultra-violet light of long wave length. By adding to a solution of carbon dioxide some substance such as sodium phenoxide or

paraldehyde which absorbs long wave-length ultra-violet light, the formaldehyde formed by the short wave-length is protected from polymerization. To photocatalyse the production of formaldehyde from carbon dioxide and water, Baly and Heilbron employ malachite green which has the same infra-red frequency as carbon dioxide and forms an additive compound with this substance ; methyl orange and *p*-nitrosodimethyl aniline may be used in place of malachite green.

A coloured substance which can photocatalyse the polymerization of formaldehyde to carbohydrate has not yet been found. It is assumed that chlorophyll is an ideal substance for photocatalysing both reactions ; it absorbs visible light from the sun and possesses the power of forming an additive compound with carbonic acid, as shown by Willstätter and Stoll ; by absorbing those rays which tend to decompose carbohydrate, the chlorophyll will, moreover, shift the equilibrium towards the side of the carbohydrate and the plant will thus be able to synthesize carbohydrate even when the concentration of carbon dioxide is low. In the opinion of Baly and Heilbron the formaldehyde peroxide compound of Willstätter and Stoll can have only a very transient existence. The formaldehyde as it is split off is instantly polymerized to a sugar which would account for the inability to identify formaldehyde in active leaves.

CHAPTER IV.

THE SYNTHESIS OF PROTEINS.

In view of our limited knowledge of the chemistry of proteins, the degree of our ignorance respecting their synthesis in plants is not surprising.

It is generally agreed that the leaves are the important centres of protein formation, and they show a periodicity in their nitrogen content. Otto and Kooper [*] and Le Clerc du Sablon [†] found that there is a gradually decreasing amount of nitrogen from the spring to the autumn, and that leaves of several different plants, even in different stages of development, exhibit a greater nitrogen content in the morning than in the evening.

The supply of nitrogen is an essential factor, and this element must be presented in a form suitable for the nutritive processes of the plant. Thus amongst the non-green plants, *Saccharomyces* is unable to make use of nitrates and but little use of simple amines; urea can be assimilated under certain conditions, but the best results obtain from the employment of peptone, a culture solution of peptone and sucrose giving 177·4 per cent increase in dry weight after the completion of fermentation.[‡]

There is, however, much variation shown by the lower non-green plants in this respect, hardly a matter for surprise in such metabolic gymnasts; *Bacillus coli* will flourish on a diet containing salts either of ammonia, of simple amides or of amino acids, whilst the cholera bacillus can apparently make no use of ammonium salts but is able to utilize amino acids. For the Fungi, Boas [§] considers that in general ammonium salts, and especially the ammonium salts of organic acids such as quinic

* Otto and Kooper: " Landwirthsch. Jahrb.," 1910, **39**.
† Le Clerc du Sablon: " Rev. Gen. Bot.," 1904, **16**, 341.
‡ Bokorny: " Chem. Zeit.," 1916, **40**, 366.
§ Boas: " Biochem. Zeit.," 1918, **86**, 110.

acid, are better than amino acids as a source of nitrogen in protein synthesis. He points out that such feeding experiments should be brief, in order to avoid secondary, especially proteolytic, changes, and that the reaction of the culture medium is all-important. Acidity may inhibit the growth of many fungi, so that if ammonium salts of inorganic acids be used as a source of nitrogen, the observed results may be due not to the inability of the plant to employ the particular salt, but to the acidity which develops as the nitrogen is assimilated.

For ordinary green plants the supply of nitrogen is found in the simple nitrogen-containing salts of the soil water. Thus the fertility of the soil, not only with respect to nitrates but also in regard to other substances,* is an important factor, conditioning the amount of protein found in the plant. In addition to nitrates, some plants can make use of ammonium salts. Hutchinson and Miller † found this to be true under conditions of culture which precluded the presence of nitrates in the soil. In this respect, however, all plants do not behave alike; whilst some will grow equally well whether supplied with nitrates or ammonium salts, others flourish best when supplied with the former, and others seemingly prefer ammonium salts to begin with and then nitrates.

Mention has just been made of the importance of soil substances other than nitrates in the protein synthesis of plants : potassium may be taken in illustration. Species of bacteria grown in the dark in culture media containing the requisite organic food materials and salts but lacking potas-sium show but poor development and no protein synthesis. Under similar cultural conditions seedlings of the beet showed seven times less protein and eighteen times less sugar, when grown without potassium, as compared with the controls ; when grown under sterile conditions in a culture medium containing a sugar and supplied with known amounts of carbon dioxide, it was found that those grown in the light were independent of potassium as regards the synthesis of protein, and that the addition of sugar to the culture medium

* Whitson and Stoddart : "Ann. Rep. Wisconsin Exp. Sta.," 1904, 193.
† Hutchinson and Miller : " Journ. Agric. Sci.," 1909, 3, 179.

resulted in an increase of protein. In darkness, on the other hand, a less vigorous development obtained and only those plants supplied with potassium salts showed protein formation.

It would therefore appear that the action of potassium is partly indirect, and only in darkness does it play an important rôle in the production of proteins. Since potassium is mildly radioactive, it may possibly serve as a source of energy in promoting the analysis of carbohydrate.[*]

These observations introduce the problems connected with the light factor. Earlier opinions were that light was an important and a direct factor in the synthesis of proteins: radiant energy is certainly of great importance in that it is a necessity for the green plant in the making of carbohydrate, which is in its turn a requisite in the formation of proteins.

The obvious essentiality of nitrogen in the building of proteins and the fact that in general the element is absorbed by the plant in the form of nitrate has been remarked upon: but nitrate as such is a relatively inert substance and does not readily lend itself to chemical change; nitrite, on the other hand, is a more labile substance.

That the plant is able to convert nitrate into nitrite was first observed by Laurent,[†] and later Irving and Hankinson,[‡] working on *Sagittaria*, came to the conclusion that nitrite must be an intermediate compound in the metabolism of nitrates.

With regard to the occurrence of nitrites in the plant, Aso [§] has established their occurrence in etiolated potato shoots. In this connexion it is of interest to note that so long ago as 1888 Schimper,[||] experimenting with cut leaves of *Sambucus* and with potted plants of *Pelargonium zonale*, found that nitrates were destroyed in green leaves exposed to daylight but were not so destroyed if the leaves were kept in the dark, and in agreement with this, shade leaves were found to be richer in nitrates than sun leaves. Furthermore, no destruction of nitrate occurs in etiolated leaves exposed to sunlight.

[*] See Stoklasa: " Biochem. Zeitsch.," 1916, **73**, 107.
[†] Laurent: " Ann. Inst. Pasteur," 1890, **4**, No. 11.
[‡] Irving and Hankinson: " Biochem. Journ.," 1908, **3**, 87.
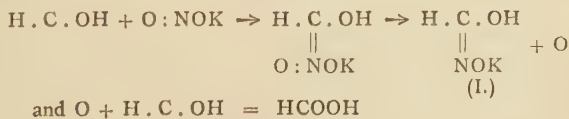[§] Aso : " Beih. Bot. Zentr.," 1903, **15**, 208, and 1914, **32**, 146.
[||] Schimper: " Bot. Zeit.," 1888, **46**, 128.

It was first shown by Thiele * that potassium nitrate, on exposure to the rays from a quartz mercury vapour lamp, was reduced to potassium nitrite with evolution of oxygen. With a view to the further study of the photochemical decomposition of nitrites, Baudisch † exposed mixtures of potassium nitrite and methyl-alcohol in aqueous solution to diffused daylight and to ultra-violet light and found that the methyl-alcohol became oxidized to formaldehyde at the expense of the nitrite which was reduced to hyponitrite, and this latter, at its moment of formation, reacted with the formaldehyde to produce the potassium salt of formhydroxamic acid (I.)

$$KNO_2 + CH_3OH = KNO + HCHO + H_2O$$
$$KNO + HCHO = H \cdot C \cdot OH$$
$$\|$$
$$NOK$$
$$(I.)$$
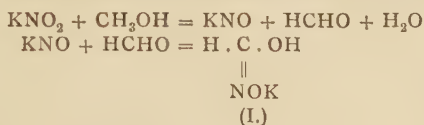
This reduction of nitrate or nitrite in presence of alcohol is a purely photochemical reaction since no such change could be produced in the dark even if the solutions were boiled.

More recently Baly and his collaborators, to whose work on carbohydrate synthesis reference has already been made, have investigated the photosynthesis of nitrogen compounds from nitrates and carbon dioxide by passing this gas through aqueous solutions of potassium nitrate or nitrite exposed to ultra-violet light.   In these circumstances activated formaldehyde is produced for which the formula H . C . OH is suggested, the activity being due to the divalent carbon.   According to Baly, Heilbron and Hudson ‡ this activated formaldehyde reacts with the nitrite to produce the potassium salt of formhydroxamic acid (I.), an atom of oxygen being evolved which oxidizes a further quantity of formaldehyde to formic acid.   These changes may be represented by the following equations :—

$$H \cdot C \cdot OH + O:NOK \rightarrow H \cdot C \cdot OH \rightarrow H \cdot C \cdot OH$$
$$\| \qquad\qquad \| \qquad + O$$
$$O:NOK \qquad NOK$$
$$(I.)$$

and $O + H \cdot C \cdot OH = HCOOH$

* Thiele : " Ber. deut. chem. Gesells.," 1907, **40,** 4914. See also Moore and Webster : " Proc. Roy. Soc.," Lond., B., 1919, **90,** 158.

† Baudisch : " Ber. deut. chem. Gesells.," 1911, **44,** 1009. See also loc. cit., 1916, **49,** 1176, and 1918, **51,** 793.

‡ Baly, Heilbron and Hudson : " Journ. Chem. Soc.," 1922, **121,** 1078.

Under the conditions of the experiment, the potassium salt is completely hydrolyzed to the free acid—

$$\begin{array}{c} H.C.OH \\ \| \\ N.OH \end{array}$$
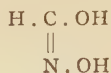
The latter compound can readily lose oxygen to produce a compound of the formula

$$\begin{array}{c} H.C.OH* \\ \| \\ N.H \end{array}$$
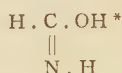
which could condense with more activated formaldehyde to produce a labile ring compound (III.)

$$\begin{array}{c} H.C.OH—H.C.OH \\ \diagdown \quad \diagup \\ NH \\ (III.) \end{array}$$
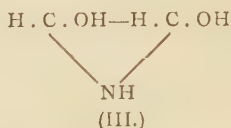
which by intramolecular rearrangement would give glycine CH_2NH_2COOH.

Evidence in support of the correctness of these views is furnished by the actual production of α-amino acids on exposing aqueous solutions of formhydroxamic acid and formaldehyde to ultra-violet light, whereby the earlier claims made by Baudisch † to have obtained substances of this nature are confirmed.

In addition, the authors claim to have produced alkaloids. The formation of these substances is explained by assuming formhydroxamic acid to condense with three or four molecules of activated formaldehyde to produce compounds (IV.) and (V.).

$$\begin{array}{c} H.C.OH—H.C.OH \\ | \qquad\qquad | \\ HCOH \qquad HC.OH \\ \diagdown \qquad\quad \diagup \\ NH \\ (IV.) \end{array}$$

$$\begin{array}{c} H.C.OH \\ \diagup \qquad \diagdown \\ HCOH \qquad HCOH \\ | \qquad\qquad | \\ HCOH \qquad HCOH \\ \diagdown \qquad \diagup \\ NH \\ (V.) \end{array}$$
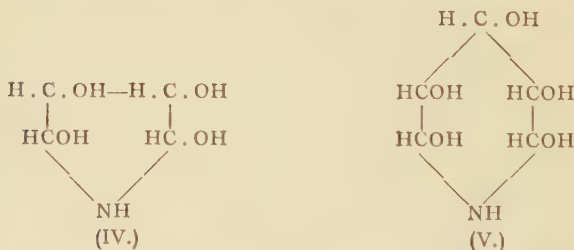
* This compound is a hydrate of hydrocyanic acid which is of significance with regard to the formation of cyanogenetic glucosides.

† Baudisch: " Zeit, angew. Chem.," 1913, **26**, 612,

which by loss of oxygen and water should give pyrrole * or pyridine compounds respectively.

By the condensation of two molecules of formhydroxamic acid with one molecule of formaldehyde, the compound (VI.) would be produced which by loss of oxygen and water would yield glyoxaline (VII.)

$$\begin{array}{cc}
\text{H . COH—NH} & \text{CH—N} \\
| \qquad | & \| \qquad \| \\
\text{HCOH HCOH} & \text{CH CH} \\
\diagdown \diagup & \diagdown \diagup \\
\text{NH} & \text{NH} \\
\text{(VI.)} & \text{(VII.)}
\end{array}$$
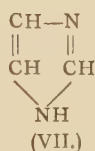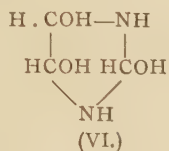
and again evidence of the actual formation of this substance is adduced, as well as of histidine,† the sustituted a-amino acid derived from glyoxaline.

The authors' interpretation of their results is best given by quoting their own words : " Our results leave no doubt that the activated formaldehyde photosynthetically produced in the living chloroplast reacts with potassium nitrite with extraordinary ease to produce formhydroxamic acid, which at once proceeds to condense with more of the activated formaldehyde to give various nitrogen compounds.   It follows from this that the synthesis of the nitrogen compounds found in the plant is not photosynthetic except in so far as the production of the activated formaldehyde by the chlorophyll is concerned.   The various amino-acids, proteins, alkaloids, etc., are natural and indeed inevitable results of the photosynthesis of formaldehyde in the presence of potassium nitrite.   Their formation has been considered by some to savour of the mysterious, the mystery being found in the question as to how a plant succeeds in synthesizing the very substances it requires for its existence.

" The life and growth of a plant consists in the utilization of the products formed in its leaves.   There is no real mystery in

$$\begin{array}{cc}
& \text{CH} \\
\text{* CH—CH} & \diagup \diagdown \\
\| \qquad \| & \text{CH CH} \\
\text{CH CH} & \| \qquad | \\
\diagdown \diagup & \text{CH CH} \\
\text{NH} & \diagdown \diagup \\
\text{Pyrrole} & \text{N} \\
& \text{Pyridine}
\end{array}$$
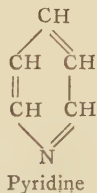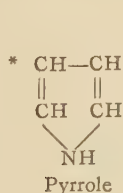
† See Vol. I,, p. 325.

the formation of these products, the plant has no choice in the matter, since with given conditions of chlorophyll, carbonic acid, light energy and potassium nitrite the synthesis must follow its natural course just as we have found to take place *in vitro.*

" A further conclusion of importance is that the region where the synthesis occurs must necessarily be restricted to the leaves.   Since it must not be forgotten that the synthesis of hexoses is taking place concurrently, the conditions are perfect for the formation of glucosides and we believe that the products of the nitrogen synthesis are translocated as soluble glucosides. The fact that nitrogen derivatives are found in other parts of the plant cannot be accepted as an argument that they must have been synthesized in those parts.   There can be no doubt that the synthesis takes place in the leaves and that the compounds are subsequently distributed as soluble glucosides by the normal translocatory processes."

Commenting on the last statement, it should be borne in mind that no evidence is adduced in support of the existence of such soluble protein glucosides.

The authors summarize their ideas in the following scheme.

Potassium nitrate   Carbonic acid
        ↓   ↓
Potassium nitrite   Activated formaldehyde
        | ↓
        ↓_____↓ |_____↓
                ↓ | Hexoses
        Formhydroxamic acid |
                ↓   ↓
                        ↓
        Nitrogen bases   α-Amino-acids
                |
        ↓_____↓
Alkaloids and xanthine   ↓
derivatives   Substituted α-amino-acids
                                (Histidine, etc.)
                                        ↓
                                    Proteins
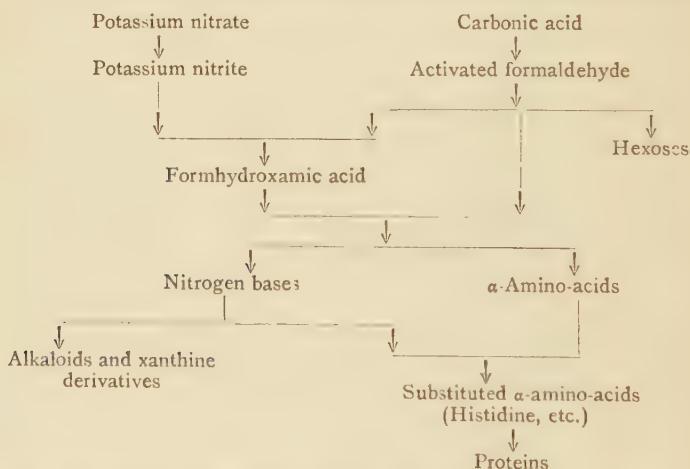
Whilst the foregoing pages have dealt with the synthesis of proteins in daylight or ultra-violet light it must be borne in mind that the synthesis of proteins can also take place in the dark and in tissues free from chlorophyll, provided that an adequate

supply of carbohydrate be at hand.* Zaleski and Suzuki †
found that the leaves of the sunflower floating upon a solution
containing sugar and nitrate produced considerable quantities of
proteins in the dark, from which it appears that nitrogen as-
similation is not a photochemical process, and that light is only
of indirect importance in providing one of the means for the
formation of carbohydrates.‡ This opinion also is held by
Loew.§

The synthesis of proteins is conditioned by the available
supply of carbohydrate, and since photosynthesis is a daylight
process, it is not surprising to find that the production of pro-
teins may be four or five times as great in the light as in
darkness.‖ Baudisch ¶ is of the opinion that the formation of
protein under abnormal conditions in the dark is no proof
that the process is not a photochemical one under normal con-
ditions; he considers that the synthesis may in this case be
due to some abnormal chemical processes which reduce the
nitrates and so aid in the production of proteins. It has also
been stated that if but small quantities of carbohydrate are
available, the synthesis of proteins, in darkness, may stop at
the formation of amides,** which some plants, e.g., Algæ such
as *Pleurococcus* and *Raphidium*, and the Fungi *Eurotium* and
Penicillium, can directly assimilate,†† an aspect of the subject
to which allusion has already been made.

There is available but little exact knowledge regarding the
specific action of different wave lengths of radiant energy
which may be concerned in the different phases of protein
metabolism. Schanz ‡‡ finds that ultra-violet light renders
proteins less soluble, possibly an account of its deleterious
action on enzymes,§§ and in this is found a reason for the

* Jost : " Biol. Centrlbl.," 1900, **20,** 625.

† Zaleski and Suzuki : " Ber. deut. bot. Gesells.," 1897, **15,** 536; " Bot.
Centrlbl.," 1901, **87,** 281 ; Suzuki : " Bull. Coll. Ag. Tokyo," 1898, **2,** 409 ; 3,
241.

‡ Zaleski : " Ber. deut. bot. Gesells., 1909, **27,** 56.

§ Loew : *id.,* 1917, **50,** 909.

‖ Montemartini : " Atti. R. Inst. Bot. Pavia," 1905, II., **10,** 20.

¶ Baudisch : " Zentr. Bakt. Parasit.," 1912, **32,** 520.

** Jakobi : " Biol. Centrlbl.," 1898, **18,** 593.

†† Lutz : " Bull. Soc. Bot. France," 1902, **48,** 118.

‡‡ Schanz : " Ber. deut. bot. Gesells.," 1918, **36,** 619.

§§ See Vol. I., p. 353.

dwarf habit of alpine plants which are subjected to a high
degree of insolation.   Bovie * draws attention to the efficiency
of the extreme ultra-violet rays, the so-called Schumann rays,
and points out that the photolysis of protein and of protoplasm
increases with the diminution in the wave length of the incident
light; in the extreme ultra-violet region, the amount of
chemical change is proportional to the product of the light
intensity and the time of exposure.

HYDROLYSIS OF PROTEIN ON GERMINATION.

The protein content of a seed is a reserve food which must
be hydrolyzed before it can be translocated and made available
for the growing parts.   The hydrolysis of proteins yields amino
acids, such as leucine, asparagine, and tyrosine, both in the
organism and in the test tube.   From the facts of animal
physiology there is no doubt that amino acids, the products of
the hydrolysis of protein food in the alimentary tract, are recon-
structed to form protein in the various tissues after their passage
through the walls of the intestine into the blood stream.   There
is reason to suppose that the same sequence obtains in the
plant.   Thus Zaleski † found that during the ripening of pea
seeds there was an increase in the amount of the protein at the
expense of the amino acids and organic bases, as indicated by
nitrogen determinations of these compounds.

	Control.		After Five Days.	
N of proteins . . .	79·2 per cent of total N		89·2 per cent of total N	
N of amino acids . .	8·7	,, ,,	4·6	,, ,,
N of organic bases . .	10·8	,, ,,	5·6	,, ,,
N of other compounds .	1·4	,, ,,	·8	,, ,,

The results were not so well marked for all seeds; thus under
similar conditions but little protein synthesis took place in the
maize, whilst in the sunflower there was a diminution of protein.

Of these dissociation products of proteins, asparagine is
amongst the more conspicuous in the plant.   It occurs in the

---

* Bovie : " Bot. Gaz.," 1916, **61,** 1.

† Zaleski : " Ber. deut. bot. Gesells.," 1905, **23,** 126; " Beih. bot.
Zentrbl.," 1911, **27,** 63.

developing parts * in greater abundance than in the members where the reserve proteins are stored ; Schulze † found that only 7·62 per cent of asparagine occurred in the cotyledons, whilst 31·81 per cent obtained in the axis of the lupin. Also the relative amounts of asparagine and aspartic acid show considerable variation during germination and, in the last stages, the amount of asparagine formed is in a proportion greater than the amount of protein decomposed. In the instance of *Cicer arietinum*, there is a marked increase in the amount of amino acids and their amides during germination, which increase is at the expense of the proteins.‡ Similarly there is an accumulation of amides in the shoots of germinating peas ; the amount of amide nitrogen in the seed leaves fluctuates much at first but in the later stages of germination there is a marked increase, whilst, concurrently, the a-amino acids decrease in amount and and finally disappear.§ From these and such like facts there is no doubt that in the plant asparagine, and possibly also other amides, have their origin in the dissociation of the already elaborated protein.

With regard to the formation of asparagine, this compound may possibly be produced by the combination of ammonia and aspartic acid ‖ to form ammonium aspartate which gives origin to the asparagine by the loss of a molecule of water. Prianichnikov,¶ whilst recognizing in ammonia the end product of protein dissociation, considers that it also is an initial stage in the formation of proteins and, according to him, asparagine is the form in which it is stored.

Sure and Tottingham,§ from the relationship obtaining between the amides and amino acids during the germination of the pea, consider that the amino acids serve for the production of amides in the plant.

Asparagine lends itself to condensation more or less readily,

---

* This may easily be shown by germinating lupin seeds in the dark until the hypocotyl is a few inches in length. On mounting a section of the hypocotyl in strong alcohol and examining under the microscope, a large number of crystals of asparagine will be seen.

† Schulze : " Landw. Jahrb.," 1878, 411.

‡ Zlataroff : " Biochem. Zeitsch.," 1916, **75**, 200.

§ Sure and Tottingham : " Journ. Biol. Chem.," 1916, **26**, 535.

‖ Schulze : loc. cit. Prianichnikov : " Ber. deut. bot. Gesells.," 1904, **22**, 35. Treboux : " Ber. deut. bot. Gesells.," 1904, **22**, 570.

¶ Prianichnikov : " Bull. Agric. Intell.," 1917, **8**, 204.

and by simply boiling a solution, the dipeptide of aspartic acid is formed.*

With regard to other views concerning the synthesis of proteins, Treub,† from his investigations on the distribution of, the periodic variation in the amount of, and cognate observations on the cyanogenetic glucosides,‡ concluded that hydrocyanic acid is the first recognizable product of nitrogen assimilation and possibly is the first organic nitrogen compound formed. Whilst on purely chemical grounds it is not impossible that acetone cyanhydrin, $CH_3COHCNCH_3$, may be a stage in protein synthesis, Treub's conclusions are not convincing : free hydrocyanic acid has not been identified in plants and its compounds may equally well result from the oxidation of amino acids : in other words, the known facts regarding compounds of hydrocyanic acid in the plant neither prove nor disprove Treub's hypothesis.§

* Ravenna and Bosinelli : " Rend. R. Acad. Lincei.," 1919, **28**, ii. 113.
† Treub : "Ann. Jard. Bot. Buitenzorg," 1895, **13**, 1 ; 1904, **19**, 86 ; 1907, **21**, 107.
‡ See Vol. I., p. 173.
§ Rosenthaler : " Schweiz. Apoth. Ztg.," 1920, **58**, 137.

CHAPTER V.

RESPIRATION.

THE maintenance of life is impossible without a supply of energy, the motive power in the absence of which activities must come to an end. This is more obvious in an animal than in a plant, which generally is less obtrusive in its movements and various activities and may make use of radiant energy, more particularly in the production of food.

That energy may be produced by the combustion of a suitable fuel is a commonplace and is illustrated in the steam engine, the boilers of which are heated by fires fed with fuel which varies according to local circumstances. The heat-producing power, or calorific value, of fuels varies and the most efficient material in this respect is the one which produces the maximum number of heat units or calories for a given weight of substance.

In all cases the heat produced in the combustion of a fuel is that due to the chemical reaction of oxidation, or, in other words, the heat given out when the constituent elements of the fuel severally combine with oxygen to form the corresponding oxides. The heat of combustion of a compound will, therefore, depend upon the heat of combustion of its constituent elements and is greater the richer the compound is in elements possessing a high heat of combustion.

Now it is a principle in physical chemistry that the heat of any chemical reaction depends solely upon the initial and final products, and the total heat evolved is the same by whatever method the final products are obtained, i.e., whether in one single process or by a series of intermediate stages, and also whether the reaction proceeds rapidly or so slowly that there is no perceptible rise in temperature. In view of these facts it will be clear that the same laws hold for the low temperature of oxidation of various oxidizable substances in the living cell as for the combustion of these substances in air or oxygen.

The heat of combustion of an element is determined by causing it to combine with oxygen in a closed chamber and measuring the heat evolved calorimetrically. The two equations—

$$C + O_2 = CO_2 + 94\cdot8K$$
$$H_2 + O = H_2O + 69K$$
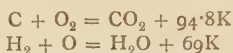
indicate that by the complete oxidation of 12 grams of carbon or 2 grams of hydrogen 94·8 and 69 kg. calories * are evolved.

Expressing the above two equations in another form—

1 gram of hydrogen on combustion yields 34·5K
1 ,, ,, carbon ,, ,, ,, 7·9K

From this it will be seen that hydrogen on combustion yields relatively much more heat than carbon; consequently compounds rich in hydrogen have a high calorific value. Moreover, since oxygen itself has no calorific value, it follows that the presence of this element in compounds reduces their calorific value. Thus it comes about that fats which contain only about 11 per cent of oxygen have a considerably higher heat of combustion than carbohydrates which contain as much as 53 per cent of this element. The actual values of the heat obtainable by the combustion of some of the more important fuel substances of the living cell are as follows:—

1 gram of carbohydrate = 4·1 calories
1 ,, ,, alcohol = 7·1 ,,
1 ,, ,, fat = 9·1 ,,
1 ,, ,, protein = 5·8 ,,

In the procurement of energy the plant exhibits a wider range than does the animal, and this to a larger extent than is often thought; thus Ramann and Bauer † have estimated that young saplings of deciduous trees may show a loss of 20 to 45 per cent of their dry weight during the burst of activity which follows the winter's sleep.

The term respiration here is used to include all those processes which involve a liberation of energy employable by the organism in its various activities. Respiration is not merely the absorption of oxygen and the excretion of carbon

* A kilogram calorie is the amount of heat required to raise the temperature of 1 kg. of water through 1° C. A statement to the effect that the complete oxidation of glucose, for example, liberates 709 kg. calories therefore means that the heat energy liberated during the combustion of 180 grams of glucose is sufficient to heat 709 kgs. of water through 1° C.

† Ramann and Bauer: "Jahrb. Wiss. Bot.," 1911, 50, 67.

dioxide as is too often supposed, an idea having its origin in the lungs of an animal being termed the organs of respiration. Respiration is essentially a catabolic process, and any organ of a plant or of an animal which is doing work is an organ of respiration in that it cannot accomplish its task without the energy obtained by the exertion of appropriate mechanisms. The lungs and the respiratory tract, on the one hand, and the stomates, lenticles, " respiratory chamber," and the intercellular space system on the other, are strictly comparable : they are organs of breathing ; structures, reservoirs, and surfaces for a preliminary of respiration, the conveyance and initial absorption of oxygen, and for the ultimate elimination of the gaseous waste of physiological combustion.

This motive power commonly is obtained by the physiological combustion of carbohydrate, by which, theoretically, a molecule of sugar is completely oxidized by 6 molecules of oxygen giving origin to 6 molecules each of carbon dioxide and water with the liberation of a considerable quantity of energy.

But oxidation is not quite the simple operation as might be supposed from the foregoing statement. Thus the expression $CO + O = CO_2$ may be a true statement of the result of the complete oxidation of carbon monoxide to carbon dioxide, but it does not necessarily represent the mechanism by which the change is effected ; as a matter of fact it has been shown by Wieland [*] and others that water is an essential in the process and the first phase is an hydration which leads to the formation of formic acid.

$$CO + H_2O = H-C \underset{\textstyle O}{\overset{\textstyle OH}{\Big\langle}}$$
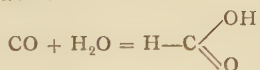
Interaction between the formic acid and oxygen then takes place, leading to the production of water and carbon dioxide,

$$HCOOH + O = H_2O + CO_2$$
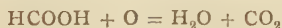
It will, however, be seen that the resultant of these two reactions is correctly expressed by the equation $CO + O = CO_2$.

From considerations such as these, together with the fact that oxidative processes enter largely into the energy-obtaining

* Wieland, " Ber. deut. chem. Gesells.," 1912, 45, 679, 2613, where earlier literature is also quoted.

processes of plants, it is desirable to examine the mechanism of oxidation somewhat closely.

THE MECHANISM OF OXIDATION.

Oxidation may be effected in one of two ways; either by the addition of oxygen to, or by the removal of hydrogen from, a given compound. Similarly reduction is regarded as the addition of hydrogen to, or the removal of oxygen from, a given compound.

The change from hydroquinone (I.) to quinone (II.) by the removal of two atoms of hydrogen may be regarded as oxidation.

(I.)   (II.)
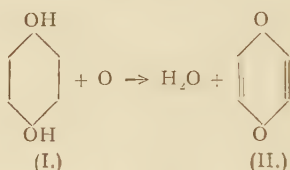
Likewise the conversion of alcohol into aldehyde, which involves the removal of two atoms of hydrogen, is an example of oxidation

$$CH_3CH_2OH + O = H_2O + CH_3CHO.$$

This conversion of alcohol into aldehyde can be catalytically effected by shaking the alcohol with palladium black in the absence of oxygen.* This is due to the affinity of palladium for hydrogen, resulting in the formation of palladium hydride, PdH_2. The reaction, however, soon comes to an end unless the hydrogen is removed; this may be effected by means of a substance, such as quinone or methylene blue, which will act as an hydrogen acceptor; if quinone is employed, this substance takes up the hydrogen, becoming reduced to hydroquinone, a colourless substance; by the use of methylene blue, however, the change is made visible since the reduction of the quinonoid methylene blue involves a loss of colour with the formation of a colourless, or leuco, compound. A more appropriate example, in that it recalls a once not uncommon laboratory experiment, is found in the changes brought about in an aqueous solution of methylene blue by the living plant: a

* Wieland : " Ber. deut. chem. Gesells.," 1912, **45**, 488, 2606.

healthy twig of *Elodea*, placed in a test tube of cold well-boiled water coloured blue with the dye, is placed in the dark ; after a time the water is quite colourless but quickly assumes its blue colour on exposure to sunlight, owing to the evolution of oxygen by the plant.   This may be explained on the assumption that during the respiration of the plant hydrogen is absorbed by the methylene blue, the hydrogen acceptor, which is thereby converted into the leuco compound.   The subsequent presence of oxygen, on exposure to light, brings about the re-oxidation of the leuco compound into methylene blue and water.

This mechanism of dehydrogenation is, according to Wieland, the one underlying many, if not all, oxidations and especially those associated with enzymes.

Reverting once more to the dehydrogenation theory of oxidation, it has been shown by Wieland that the oxidation of aldehyde to acid may be explained on the same principle, since palladium black has no action whatever upon the dry aldehyde.   This is explained by the fact that the dry aldehyde has not in itself any hydrogen for activation but that in the presence of moisture it forms the hydrate

$$CH_3C{\diagup}^O_{\diagdown H} + HOH = CH_3C{\diagup}^{OH}{\diagdown}^{-OH}_H$$

Palladium black can then activate two hydrogen atoms in this latter compound and these are then removed by a suitable hydrogen acceptor :—

$$CH_3C{\diagup}^{OH}_{\diagdown O{\overline{|H \atop H|}}} \rightarrow CH_3C{\diagup}^{OH}_{\diagdown O} + H_2$$
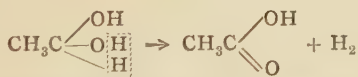
This view is supported by the fact that whereas chloral, CCl_3CHO, is unacted upon by palladium and methylene blue, that well defined substance chloral hydrate, $CCl_3CH(OH)_2$, is at once oxidized.   Further corroborative evidence concerning the essential part played by water is provided in the fact that even such a well marked aldehyde-oxidizing agent as silver oxide has no action upon dry aldehyde but oxidizes it readily when wet.

$$CH_3C{\diagup}^O_{\diagdown H} + HOH = CH_3C{\diagup}^{OH}{\diagdown}^{-OH}_H + Ag_2O = CH_3COOH + 2Ag + H_2O$$
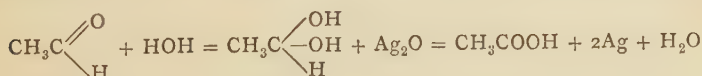
Wieland * also effected the oxidation of glucose to carbon dioxide by shaking it with palladium black and methylene blue at room temperature. He similarly effected the oxidation of phenolic substances such as hydroquinone and pyrogallol to quinone and purpurogallin respectively in the entire absence of oxygen, oxidations which, according to the Bach and Chodat theory,† are effected by atmospheric oxygen activated by an oxidase system.

Wieland ‡ also investigated the so-called Schardinger reaction in milk. This reaction, designed by Schardinger to distinguish boiled from unboiled milk, depends upon the fact that unboiled milk when warmed with methylene blue and a drop of acetic aldehyde decolorizes the dye, whereas boiled milk produces no such change. According to Wieland this action is due to an enzyme, for which he proposes the name dehydrase ; this enzyme dehydrogenates the aldehyde hydrate in the same way as the palladium black, the methylene blue again acting as hydrogen acceptor. The same enzyme is also able, by slightly varying the conditions, to produce from two molecules of salicylic aldehyde one molecule of the corresponding acid and one molecule of the alcohol ; the latter being produced by one of the molecules of salicylic aldehyde itself acting as the hydrogen acceptor.

$$C_6H_4OH . CHO + H_2O \rightarrow \left[C_6H_4OH . C{\overset{\displaystyle OH}{\underset{\displaystyle OH}{-OH}}} \right] \rightarrow C_6H_4OH . C{\overset{\displaystyle OH}{\underset{\displaystyle O}{\diagdown}}} + 2H$$

A second molecule of salicylic aldehyde then acts as hydrogen acceptor forming a molecule of salicylic alcohol

$$C_6H_4OH . CHO + 2H \rightarrow C_6H_4OH . CH_2OH.$$
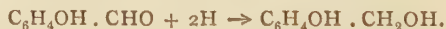
This change effected by enzyme activity of two molecules of an aldehyde into one molecule each of the corresponding alcohol and acid was originally thought by Parnas § to be due to a special enzyme to which he gave the name aldehyde mutase ; Wieland's work, however, shows that this substance is none other than dehydrase. This same enzyme dehydrase can be

* Wieland: " Ber. deut. chem. Gesells.," 1913, 46, 3331.
† Vol. I., p. 396.
‡ Wieland: " Ber. deut. chem. Gesells.," 1914, 47, 2085.
§ Parnas : " Biochem. Zeitsch.," 1910, 28, 274.

invoked to explain the reducing action of an aqueous extract of potato. The existence of this was first demonstrated by Bach in the following simple experiment. One gram of freshly pounded potato is heated in a test tube to 60° C. with 10 c.c. of 4 per cent aqueous solution of sodium nitrate together with 3 drops of 10 per cent solution of acetic aldehyde. After two minutes the solution gives a strong reaction for nitrite by the Griess Ilosvay reagent. The reduction of the nitrate to nitrite was thought by Bach to be due to the activity of a per-hydridase existing in the potato together with a per-oxidase system. According to Wieland, however, this is simply explained by the dehydrase activating the hydrogen of the aldehyde hydrate whilst the sodium nitrate acts as the hydrogen acceptor.

$$\text{1. } CH_3C\diagdown{}_{H}^{O} + HOH = CH_3C\diagdown{}_{H}^{OH}{-}OH$$

$$\text{2. } CH_3C{-}{}_{H}^{OH}CH \rightarrow CH_3C\diagdown{}_{O}^{OH} + 2H$$

$$\text{3. } NaNO_3 + 2H = NaNO_2 + H_2O.$$
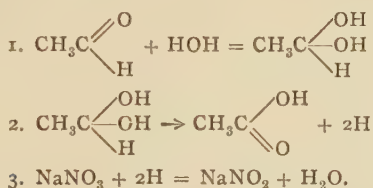
Even the biological conversion of alcohol into acetic acid by the vinegar plant, *Bacterium aceti*, was shown by Wieland to be a dehydrase action which could be effected in an atmosphere of nitrogen by leaving freshly washed cultures of the plant in contact with alcohol and methylene blue in a flask from which air had been displaced by nitrogen. In a comparatively short time the methylene blue was decolorized and after some days a measurable quantity of acetic acid had been produced.*

There is thus considerable ground for regarding biological oxidations as being primarily due to enzymes such as de-hydrase which activate hydrogen in the oxidizable substances so that it may be removed by a suitable hydrogen acceptor, which may be atmospheric oxygen. Wherefore the oxidizable substance is to be regarded as a potential hydrogen donator, and, in fact, Thunberg † goes so far as to regard hydrogen as the essential fuel of the living cell and regards only those

* Wieland : " Ber. deut. chem. Gesells.," 1913, 46, 3336.
† Thunberg : " Skand. Archiv. Physiol.," 1920, 40, 1.

substances as possible intermediate metabolites which when left in contact with methylene blue in the absence of oxygen decolorize this substance and therefore act as hydrogen donators. His technique consists in placing the material under examination in a test tube with freshly washed frog's muscle, to supply the dehydrase, and methylene blue, filling the tube with boiled water, and leaving the whole in a thermostat and examining at intervals. If the methylene blue is decolorized, the substance in question is a hydrogen donator and consequently a possible intermediate metabolite.

A highly important contribution to the mechanism of oxidation in the living cell is furnished by the discovery and isolation by Hopkins * of a dipeptide composed of cystein and glutamic acid to which he gives the name of glutathione. This substance can act alternatively as an hydrogen acceptor or as an hydrogen donator, according as it exists in the oxidized or the reduced form. This will be intelligible from an examination of the accompanying formulæ :—

$$
(a)\ 2\ \begin{array}{c} CH_2 . SH \\ | \\ CHNH_2 \\ | \\ COOH \end{array} \quad -2H \rightarrow \begin{array}{c} CH_2 . S . S . CH_2 \\ | \qquad\qquad | \\ CHNH_2 \quad CHNH_2 \\ | \qquad\qquad | \\ COOH \quad COOH \end{array}
$$

$$
(b)\ \begin{array}{c} CH_2 . S . S . CH_2 \\ | \qquad\qquad | \\ CHNH_2 \quad CHNH_2 \\ | \qquad\qquad | \\ COOH \quad COOH \end{array} + 2H \rightarrow 2 \begin{array}{c} CH_3SH \\ | \\ CHNH_2 \\ | \\ COOH \end{array}
$$
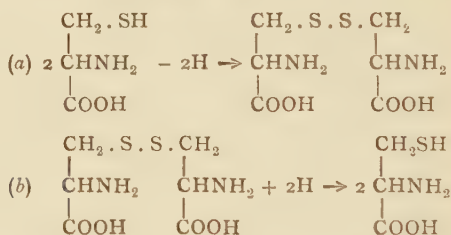
From these formulæ the glutamic acid residues have been omitted for the sake of simplicity ; the mode of action of the dipeptide as hydrogen donator in the first instance and as hydrogen acceptor in the second is obvious ; in other words, this substance may act alternately as an oxidizable reducing substance and as a reducible oxidizing agent. Glutathione is not uncommon in plant tissues although the coloration by which it is recognized usually is far less intense in vegetable than in animal tissue. Its presence in yeast may be demonstrated by grinding the cells in a mortar with a little sand and some saturated solution of ammonium sulphate. On pouring off and adding to the supernatant liquid a few drops of

* Hopkins : " Biochem. Journ.," 1921, 15, 286.

5 per cent solution of sodium nitroprusside and a little strong ammonia, a pink colour is produced. This colour reaction, which also is given by a number of other reducing substances such as aldehydes, acetone, hydrogen sulphide, etc., is only given by the reduced or cysteine form of the dipeptide and not by the cystine or oxidized modification. The isolation of the pure substance from yeast in a yield of about 0·1 to 0·15 gram per kilo has also been effected by Hopkins.

The existence of a somewhat similar system of alternatively oxidized and reduced materials in the plant have been postulated by Palladin and termed by him respiratory pigments; these will be considered later. (See p. 103.)

The existence in organic tissues and fluids of the enzyme catalase, whose characteristic property is the destruction of hydrogen peroxide with the evolution of oxygen, has been known for some time. Wieland * recently has drawn attention to observations by Lesser,† Rywosch,‡ and Jörns § that anaerobes such as *Bacillus tetanus* and *B. botulinus* are deficient in catalase whilst aerobic organisms such as *Pneumococcus* and *Sarcina*, and facultative aerobes such as yeast contain catalase in quantity. It is considered that the occurrence of catalase in those cells and tissues which require oxygen is necessary for the two-fold purpose of preventing the accumulation of hydrogen peroxide which is toxic to the cell, and also for the liberation of oxygen. Hydrogen peroxide is regarded as the first product of the oxidation of hydrogen by oxygen which acts as the hydrogen acceptor in the cell,

$$O_2 + 2H = H_2O_2$$
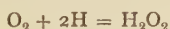
The catalase then acts upon the resulting hydrogen peroxide, breaking it up into water and oxygen, which latter is then available for further oxidation,

$$H_2O_2 = H_2O + O$$

This cycle of changes does not occur in anaerobic oxidations, which may explain the absence of catalase under these conditions.

* Wieland: "Ber. deut. chem. Gesells.," 1921, 54, [B], 2353.
† Lesser: "Zeitch. Biol.," 1906, 48, 1.
‡ Rywosch: "Zentr. Bact.," 1907, 44, 295.
§ Jörns: "Archiv. d. Hyg.," 1908, 67, 134.

Reductive processes also may result in the liberation of energy fit for use by the plant ; thus the fermentation of sugar by yeast, in which process the carbohydrate is converted into alcohol and carbon dioxide without the agency of atmospheric oxygen,* is a case in point.

The energy freed in these processes is derived from the molecular energy of the substances disintegrated and is generally expressed in terms of heat units. Thus the complete oxidation of 1 gram molecule of maltose liberates 1351 kg. calories.

The fermentation of sugar by yeast also is accompanied by the evolution of heat, although to a much lesser extent, a gram molecule of maltose yielding 42·7 kg. calories, taking Brown's † value of the heat of fermentation of 1 gram of maltose as being 125 calories. The alcoholic fermentation of sugar is therefore a wasteful process for obtaining energy when compared with oxidation, since by its means nearly thirty-one times as much sugar must be consumed to obtain as much energy as is yielded by the direct oxidation of sugar. The significance of this figure of the heat of fermentation of maltose may be realized by Brown's observations that between the temperatures of 14° and 16° C. the time required by a yeast cell to ferment its own weight of sugar varies from eighteen and a half to nineteen and a half hours and that the heat generated during one hour is sufficient to raise the temperature of the cell by 15° or 16° C. From such observations Brown estimates that at 30° C. yeast can ferment its own weight of maltose in 2·2 hours and the potential rise in temperature in the cell in one hour will be 75·5° C. under adiabatic conditions, figures indicative of an intense metabolism and, apparently, a great waste of energy. Brown suggests that the explanation for this waste is to be found in the fact that brewers' yeast is a cultivated plant grown under unnatural conditions. The wild yeasts lead a quiet life on the skin of, say, the grape : rupture of the skin of the fruit provides a nutrient medium eminently suitable for growth and reproduction accompanied by a free access of oxygen. A period of intense activity immediately supervenes and budding takes place, under the continued action of the oxygen, with

* Vol. I., p. 377.   † Brown : " Ann. Bot.," 1914, 28, 197.

remarkable rapidity : this activity necessitates a continuous supply of energy, which is provided by fermentation. Reproduction and fermentation thus are correlated. " That we can by means more or less artificial keep the reproductive power of a yeast in abeyance, whilst still availing ourselves of its fermentative power, has hitherto obscured the relation of the two functions, and hence has given rise to the somewhat exaggerated idea of the purposeless and prodigal waste of the yeast cell regarded as a living unit."

In view of the definition given above, it is obvious that any process, oxidative or reductive, which liberates energy available for use by the plant is to be included amongst respiratory processes, irrespective of the initial products consumed and the final products evolved. Thus, in addition to the oxidative processes of the higher plants in which fats, carbohydrates, proteins, and protoplasm may be physiologically consumed, the diverse metabolic processes of bacteria and comparable organisms in reducing sugar to alcohol, sulphate to sulphide, or oxidizing alcohol to acetic acid, lactose to lactic acid, ammonium salts to nitrites, nitrites to nitrates, and so on, are all processes of respiration, notwithstanding the fact that many of these activities may be extra-cellular. For green plants, oxygen is a common essential, although, as is well known, certain organisms, such as the lactic bacteria, can only flourish in the absence of oxygen whilst others, although oxygen is essential normally, have the faculty of tiding over a period of its absence. Hence respiration may be distinguished as aerobic, anaerobic,* and facultative anaerobic. The final waste products are diverse and depend upon the chemical nature of the material consumed and the method of its physiological combustion, whether by aerobic or anaerobic means.

The ordinary green plant, and certain non-green plants, in their respiration absorb oxygen and ultimately give off carbon dioxide and water. This respiration is unceasing and is continued in all living members whether active or passive until death ensues. If oxygen be entirely withheld, growth, movement, irritability and activity in general ultimately

---

* The use of the term, " intra-molecular respiration " for anaerobic respiration is wrong since all forms of respiration are essentially intra-molecular.

will come to an end.* Between the oxygen absorbed
and the carbon dioxide evolved there is maintained a cor-
relation sometimes so close as to provide a well-known
avenue for investigating certain aspects of the respiratory
processes.† This correlation, first appreciated by de Saussure,
is commonly called the respiratory quotient in the considera-
tion of which it is well to realize that it has but little value
in indicating the essential parts of the process, correlating
as it does merely a final product, carbon dioxide, of a long
series of changes with the initial oxygen, the two being but
remotely related. The ratio CO_2/O_2 is variable not only in
different plants but also in the same plant at different phases
of its existence ; in other words, the value of the ratio is
subject to the conditioning factors. Puriewicz,‡ for instance,
found that the amount of carbon dioxide evolved showed a
much greater range in variation than did the absorbed oxygen
in the same plant under different conditions, the figures ob-
tained in a series of experiments showing a variation of – 14
to 120 per cent of the average for carbon dioxide, whilst the
oxygen varied from 0 to 48 per cent of the average. Ruby §
observed in the olive that the ratio remained practically
constant and was but little affected by the age of the plant
or of the organ examined ; generally it is rather higher in
leaves from fruiting branches, a fact possibly connected with
the greater abundance of available food, and in the early
season of the year it is less than unity but later rises to unity.
As will be seen later, the carbon dioxide evolved may have
different origins ; in those instances in which it is due to the
immediate physiological combustion of available substances,
it will be apparent that the respiratory quotient will vary
according to the nature of this food.

Thus, in general terms, if sugar be the immediate respir-
able substance, the respiratory quotient will be in the neigh-

* The irritability of plants is outside our present consideration : an intro-
duction to the problems regarding the minimum pressure of oxygen necessary
to maintain movements, the streaming of protoplasm in chlorophyll-containing
cells in darkness and in an atmosphere free from oxygen, and similar subjects
will be found in the larger text books on general plant physiology.
† See Bonnier and Mangin : " Ann. Sci. Nat. Bot.," 1884, vi., 19, 217 ;
1885, vii., 2, 315 ; 1886, vii., 3, 5.
‡ Puriewicz : " Jahrb. Wiss. Bot.," 1900, 35, 573.
§ Ruby : " Ann. Sci. Nat. Bot.," 1917, 20, 1.

bourhood of unity; on the other hand, if fat be so consumed, the ratio will be less than unity. To take actual figures: the value of CO_2/O_2 for germinating oats, which are starch-containing, and for germinating mustard, which contain fat, determined by physical methods was found to be ·99 and ·92 respectively.

Although carbon dioxide represents a final product of the complete respiration of carbon compounds, other substances may be end products if physiological combustion be incomplete. Succulent plants provide instances of this; members of the Crassulaceæ do not give off carbon dioxide when first placed in darkness although the absorption of oxygen is active. There is, however, an accumulation of organic acids, malic and oxalic, and it is not until these have accumulated in relatively large quantities that carbon dioxide is evolved as in a normal plant.* The peculiar metabolism of these plants is generally associated with and explained by their massive structure rendering the movement of gases a relatively slow process. In other words, the catabolism stops at an organic acid stage which forms a reserve of carbon dioxide which is rendered available for carbon assimilation when the acids are decomposed under photosynthetic conditions. There is thus a conservation of carbon dioxide. Comparable phenomena occur in bacteria under certain conditions, the formation of acids being a well-known occurrence in their oxidative activities.

Of other products of respiration, carbon monoxide has been described by Langdon and Gailey in the pneumatocysts of *Nereocystis Luetkeana*,† a unique example as far as is known. The bladders contain an atmosphere of nitrogen, oxygen, carbon monoxide, but no carbon dioxide. An analysis of the gaseous contents of over a thousand of these floats showed the carbon monoxide to vary from 1 to 12 per cent by volume, whilst the oxygen ranged from 15 to 25 per cent. Only when oxygen is present does carbon monoxide form in the bladders; if the oxygen be replaced by nitrogen or hydrogen, no carbon monoxide results. The gas is produced naturally both by day and by night but it is not formed

* See Nicolas: "Compt. rend.," 1918, **167**, 131.

† Langdon: "Journ. Amer. Chem. Soc.," 1917, **39**, 149; Langdon and Gailey: "Bot. Gaz.," 1920, **70**, 230.

when the plant is ground and allowed to undergo autolysis or decay, under which circumstances carbon dioxide and hydrogen are produced. From these facts it is concluded that the gas is a respiratory product and has no connection with carbon assimilation.

The accumulation of the products of physiological combustion will bring about a modification, if not a complete cessation, of the process which will lead to the termination of other activities. Thus rotation of protoplasm in the cells of *Elodea* will come to an end in the presence of an undue amount of carbon dioxide. The germination of seeds is retarded or inhibited by high partial pressures of carbon dioxide in the atmosphere : this inhibition may remain in force only so long as the seeds are exposed to the enriched atmosphere, germination taking place after removal to a normal atmosphere as in the bean, cabbage, barley, pea, and onion ; or, the inhibition may continue indefinitely after removal to normal surroundings, germination only taking place after complete drying and re-wetting or by the removal of the testa as in *Brassica alba.* The degree of increase in the partial pressure of carbon dioxide required to effect inhibition of germination varies for different plants, and the retardation of germination depends on the time of exposure and the character of the seed. Similarly the sprouting of a potato is inhibited by an increase of 20 per cent in the carbon dioxide of the atmosphere. A higher concentration causes marked injury and ultimately death.*

These facts are of considerable importance not only as regards the economic aspect but also in their bearing on experimental work : results obtained for subjects contained in closed vessels, as is not infrequent in experiments on respiration, in which the products of the oxidative processes accumulate, may be an expression of the plant's activity not in a normal but in a pathological condition and, therefore, may be valueless.

INTENSITY.

In general terms, the more active the body the more intense the respiration, provided that the conditioning factors such as

* Kidd : " Proc. Roy. Soc.," Lond., B., 1914, 87, 408 ; " New Phytol.," 1919, 18, 248.

temperature, food, facility of gaseous exchange and circulation in the plant, and so on, are favourable.   Considering the plant as a whole, Bonnier and Mangin * recognize two respiratory maxima in its seasonal development, the first at germination, or on the unfolding of the leaf buds, and the second at the opening of the flower buds.   Ruby † found that the intensity of respiration was greater in leaves of young than of old plants ; thus the amount of carbon dioxide evolved per hour per gram of fresh weight of leaves from trees one year old, three years old, and many years old was respectively ·200 c.c., ·150 c.c., and ·100 c.c.   In all cases the growth period showed a one-and-a-half to two-fold increase in respiration as compared with the non-growing periods.   Nicolas ‡ compared the respiration of the vegetative parts of annual, bienniel, and perenniel plants and found that leaves and portions of stems of the same branch varied according to their age, those from the apical regions showing a three to seven-fold intensity of respiration as compared with similar structures from the basal parts.

These observations have been confirmed and extended by Kidd, West, and Briggs § who have studied the respiration of *Helianthus annuus* both in the laboratory and in the field.   They point out that the factors which may affect the rate of respiration per unit of dry weight of tissue are the concentration of the respirable material, the concentration of oxygen, the temperature, and the effective amount of respiring cell matter per unit of dry weight.   This last is the " internal " factor, the resultant of many factors, none of which as yet fully understood and some of which probably not yet formulated.   The internal factor can only be accurately measured when the other factors are not conditioning respiration.   For purposes of measuring its effect, Kidd, West, and Briggs employ a respiratory index which is the respiration, measured by the rate of carbon dioxide produced, per gram of dry weight at $10°$ C. when the amount of respirable material is not limiting and when the external concentration of oxygen is that of the atmosphere. From a large number of observations they conclude that the

* Bonnier and Mangin : " Ann. Sci. Nat. Bot.," 1885, 5, 315.
† Ruby : *Id.*, 1917, 20, 1.
‡ Nicolas : " Rev. gén. Bot.," 1918, 30, 209.
§ Kidd, West, and Briggs : " Proc. Roy. Soc.," Lond., B, 1921, 92, 368.

respiratory index of the entire plant continuously declines with increasing age. For example, entire plants 2 days from germination gave 3 mg. carbon dioxide per gram of dry weight per hour, whilst plants 136 days from germination yielded but ·39 mg. carbon dioxide per gram of dry weight per hour. A similar decrease in the respiratory index is exhibited by the stem, leaves and flowers. In the stem the value fell from ·8 mg. on the thirty-sixth day from germination to ·08 mg. on the 136th day from germination; during the same period the measure for the leaves decreased from 1·56 to ·44 mg. The fact that the initial respiratory index of successive leaves decreases with the age of the plant indicates a respiratory decrement of the merismatic tissue with age and from this it follows that the fall in the respiratory index of the whole plant is not due to the proportionate increase with age of dead tissue, sclerenchyma and tracheæ for instance.

Nicolas * also found that the presence of anthocyanins was a factor, or rather the symptom of an internal factor, of importance. Leaves containing a red pigment either as a youth form or as a permanent character absorbed more oxygen and exhibited an increased respiration as compared with green leaves, whilst leaves turned red either by accident or by stress of conditions † showed a decreased respiration as compared with green leaves, the amount of carbon dioxide being smaller.

The intensity of respiration commonly is measured by the amount of an end product given off in unit time : in aerobic respiration the final product measured is carbon dioxide or, in special cases, temperature is measured.

With regard to the measurement of carbon dioxide, it is clear that the results obtained may not be a true expression of the respiratory activity since the exhalation of the gas may be greatly hindered by various circumstances. The surface of the respiring organ is one such : Hoffmann ‡ found that the amount of carbon dioxide evolved in twenty-four hours per kilogram of large, medium, and small-sized potatoes was 259, 314, and 326 mg. respectively ; barley gave confirmatory results. He also found that the nitrogen content was im-

* Nicolas : " Compt. rend.," 1918, **167,** 131.

† See Vol. I., p. 251.

‡ Hoffmann : " Journ. f. Landw.," 1916, **64,** 289.

portant. Further, a depression of the carbon dioxide output is associated with succulent plants, hence in order to obtain a figure representing a true measure of the activity of respiration, the increase in organic acid content in addition to the gaseous carbon dioxide should be determined. This aspect is considered by Maige and Nicolas,* who point out that in the flowers they examined, the respiration intensity increased with age when stated in terms of gaseous exchange, but showed a decrease with age when measured in terms of wet and dry weight. In the case of the gynæcium, that shows, as might be expected from the activity of the contained structures, a real increase with age, whilst the other parts of the flower exhibit a decreasing respiration with age.

With regard to the heat of respiration, it is easy to demonstrate by relatively simple means, due precautions being taken against loss, that the evolution of heat is a concomitant of respiration. The temperature attained is cumulative and a remarkable rise may result which may be realized by thrusting the naked arm into a barrow load of fresh lawn cuttings, especially if there is a good admixture of clover. Exact measurements have been made by various investigators : Molisch † found that the bulked leaves of *Carpinus betulus* reached a temperature of 51° C. in fifteen hours, a fall then took place so that at the end of forty-eight hours the temperature was 34° C. After the lapse of one hundred and four hours a secondary maximum at 47° C. was attained, the temperature again showed a fall to 31° C. after one hundred and eighty hours. Of these two maxima, the first is an expression of the true respiration intensity of the leaves, whilst the cause for the second is to be found in bacterial activity. Pierce ‡ found that in germinating peas the greatest average gain in heat was 923·9 calories accumulated in 23·5 hours, which is about equivalent to 8·55 calories per minute per kilogram of peas, a measure roughly one quarter less than the amount of heat given off by a mouse under similar experimental conditions. It was further found

* Maige : " Rev. gén. Bot.," 1907, 19, 1 ; " Ann. Sci. Nat. Bot.," 1911, 14, 1. Maige and Nicolas : " Rev. gén. Bot.," 1910, 22, 409.

† Molisch : " Bot. Ztg.," 1908, 66, 211.

‡ Pierce : " Bot. Gaz.," 1912, 53, 89 ; see also Bonnier : " Ann. Sci. Nat. Bot.," 1893, 18, 12.

that the amount of heat liberated by germinating peas decreased
with age.

Since respiration is a means of obtaining energy for the
needs of the plant, the evolution of heat represents excess of
energy and is a waste product, for which reason the temperature
of a normally respiring plant is not by itself a sure guide to the
amount or intensity of physiological combustion but rather a
measure of the inefficiency of the organism.

STIMULATION.

The stimulation of the plant accelerates respiration, which
acceleration is marked by a rise in the output of carbon
dioxide and generally also by a rise in temperature.   This is
particularly true for the stimulus provided by wounding ; as
F. F. Blackman said on a certain occasion, " Precisely the
same effect is produced by peeling a potato as by flaying
a saint."   This intensification of respiration resulting from
traumatic stimulation long has been known and now is not an
uncommon laboratory exercise.   Richards * found a gradual
rise in temperature following the stimulus, attaining its
maximum about twenty-four hours after the infliction of the
injury.   In massive tissues the effect is local, but in less
compact structures, an onion bulb for example, the rise in
temperature may be demonstrated over a more extensive
area.   The rise may be two or three times as large as the
difference between the normal temperature of a potato and that
of the surrounding air.   The precise increase in respiration
depends on the extent of the injury and the nature of the tissue
operated on.   With regard to the output of carbon dioxide,
there is an initial outburst followed by a fall, a feature not at
all uncommon under drastic stimulation, and the absorption of
oxygen is rather greater than the amount theoretically required
for the quantity of carbon dioxide evolved.   Richards con-
siders that the initial outburst of carbon dioxide in part is due
to the release of the gas normally enclosed within or absorbed
by the tissues, the "residual" carbon dioxide which normally
is not exhaled.

With regard to other forms of stimulation, White † found
that pollination produces a rapid increase in respiratory activity

* Richards : " Ann. Bot.," 1897, 11, 29.   † White : *Id.*, 1907, 21, 487.

and affects the CO_2/O_2 ratio which generally is greater in pollinated carpels as compared with unpollinated gynæcia. The most striking instance was afforded by the Pelargonium, the pollinated carpels of which evolved from five to eight times as much carbon dioxide as the unpollinated.

Mention also may be made of Schley's [*] observations who found that the respiration of a geotropically stimulated root is greater than that of an unstimulated root, the respiration rate of the convex side being greater than that of the concave side during the period of perception and response.

THE ACTION OF ANÆSTHETICS.

The action of anæsthetics on the output of carbon dioxide is partly stimulatory and partly narcotic. Irving [†] found that the effect of a single dose depended on its strength: a dose of ·1 c.c. of chloroform in 970 c.c. of air brings about an immediate rise in the output of carbon dioxide, this effect subsequently disappears and the leaves then evolve as much carbon dioxide as in normal respiration. In medium doses, ·2 c.c. of chloroform in 970 c.c. of air, there obtains the same initial outburst of carbon dioxide which falls away more quickly than in the case of a small single dose and for a time remains below normal; after about six hours recovery is complete and the evolution of carbon dioxide is normal. With a dose of 1 c.c. of chloroform in 970 c.c. of air, the initial outburst of carbon dioxide is earlier and its curve is steeper, the production of carbon dioxide slowly diminishes and there is no recovery. After a strong dose of chloroform, 10 c.c. in 970 c.c. of air, there is no detectable initial outburst and the carbon dioxide output quickly falls to zero. The administration of a continuous dose of chloroform produces the same effect as a single dose two or three times as strong. Thoday [‡] likewise found that in the instances of *Prunus laurocerasus*, *Helianthus tuberosus* and *Tropæolum majus* that a small dose of chloroform leads to an immediate stimulation of respiration, the evolution of carbon dioxide and the absorption of oxygen increasing in like proportion, which indicates that the two are co-ordinated. If, however, the dose

[*] Schley : " Bot. Gaz.," 1920, 70, 69.
[†] Irving : " Ann. Bot.," 1911, 25, 1077.
[‡] Thoday : *Id.*, 1913, 27, 697.

is sufficiently large to effect a visible disorganization, such as change in colour,* there is an initial outburst of carbon dioxide then a fall to a very low level and the absorption of oxygen no longer shows any co-ordination with the amount of carbon dioxide evolved. The absorption of oxygen in some way is connected with the cell contents, especially tannin. In the *Tropæolum* leaf, which is free from tannin, the absorption of oxygen is much lower than the output of carbon dioxide, whereas in the cherry laurel and the artichoke, both of which contain tannin, the initial up-take of oxygen is very rapid, then it declines but remains at a level much higher than the output of carbon dioxide.

Similar observations have been made by others, thus Thomas † found that wheat subjected to the action of ether shows an increase of respiration followed by a decrease. Exposure to 7·3 per cent of ether is only stimulatory provided it be of short duration, an exposure of more than thirty minutes resulting in death. Similarly the main observations of Thomas have been corroborated and extended by Smith ‡ who used the hydrogen ion concentration method in finding the rate of production of carbon dioxide in wheat seedlings. It was found that the first effect of ether, used in concentrations of 1 per cent, 3·65 per cent and 7·3 per cent, was to depress the rate of respiration ; this was followed by a rapid increase to above the normal rate, which was in turn followed by a decline to much the same level in all concentrations of anæsthetic in times varying with the dose employed ; the stronger the solution of ether used, the quicker the fall.

Irwin § found in the instance of the corolla of *Salvia* that the effect of a high concentration of ether is an increased intake of oxygen and output of carbon dioxide whilst at the same time the acidity of the cell sap is reduced. With regard to the lower plants, the action of anæsthetics results in reactions similar to those exhibited by higher plants under like treatment.

---

* The change in colour is due to the oxidation of tannins and glucosides by appropriate enzymes, oxidases for example (see Vol. I., p. 392). It may be conveniently seen by exposing the flowers of the white lilac to the fumes of phenol under a bell glass.

† Thomas : " Journ. Gen. Physiol.," 1918, **1**, 203.

‡ Smith : *Id.*, 1921, **4**, 157.   § Irwin : *Id.*, 1909, **1**, 399.

Gustafson * found that *Aspergillus niger,* subjected to the action of ether, acetone, or formaldehyde, exhibits an increase in its respiratory activity followed by a decrease. The use of caffeine in saturated solution similarly brings about an increase followed by a decrease whilst a ·5 per cent solution results in a decrease of respiration intensity.

Similar results were found by Brooks † to obtain in the case of *Bacillus subtilis,* for which plant ether is toxic in low, ·037 to 1·1 per cent, and in high, 3·65 to 7·3 per cent, concentration. In strengths intermediate to these the drug acts as a stimulant to growth. Further, the action of ether varies according to the presence or absence of other substances : thus whilst the respiratory activity may be fifty times as great as the normal under the action of a 7·5 per cent solution of ether, this high intensity is considerably reduced if ·85 per cent of sodium chloride be added.

According to Haas,‡ small doses of anæsthetics are without result in the respiration of *Laminaria,* doses sufficiently large to produce a measurable result bring about a prolonged increase in respiration, whilst strong doses result in an initial increase followed by a decrease ultimately to zero. In no experiment was it found that the initial effect of an anæsthetic was a lowering of the respiration intensity from which it is concluded that the state of anæsthesia is not brought about by the lowering of the respiration. It is clear from these results that in general anæsthetics have a twofold action : a small dose is a stimulant, a large dose is a narcotic. And this is true not only for respiration but also for other expressions of plant activity such as the circulation of protoplasm within the cell.

THE CONDITIONING FACTORS.

It will be obvious from the foregoing general consideration that the intensity of respiration is dependent on the interaction of various conditioning facts the more conspicuous of which may now be examined.

TEMPERATURE.—In general terms an increase in temperature results in an increase in the respiratory activity until death

* Gustafson : " Journ. Gen. Physiol.," 1909, **1**, 181.
† Brooks : *Id.,* 1909, **1**, 193.
‡ Haas : " Bot. Gaz.," 1919, **67**, 377.

takes place. The following figures, given by Matthæi,[*] represent the mean value of carbon dioxide evolved by 2 grams of cherry laurel leaf per hour at the temperature specified :—

5·8° C.	.	.	·0001CO_2	18·2° C.	.	. ·0004$\frac{1}{2}CO_2$
14·2° C.	.	.	·0002$\frac{1}{2}CO_2$	25·7° C.	.	. ·0006CO_2
14·3° C.	.	.	·0003CO_2	29·2° C.	.	. ·0008$\frac{1}{2}CO_2$
18·1° C.	.	.	·0004CO_2	33·1° C.	.	. ·0013CO_2

The inspection of these figures shows that for the low and medium temperatures employed there is a gradual increase in the respiration activity. At higher temperatures variation obtains and it was found that the respiration intensity rapidly changed with the time of exposure and that leaves under precisely the same external conditions gave varying results due to some internal factor. This is illustrated by the following results of certain experiments on isolated cherry laurel leaves exposed to light and carbon assimilating. The respiration values represent the carbon dioxide given off by 2 grams of leaf per hour :—

38·3° C.	.	.	·0020$\frac{1}{2}CO_2$	40·9° C.	.	. ·0014CO_2
38·3° C.	.	.	·0023CO_2	40·9° C.	.	. ·0016CO_2
			42·9° C.	.	. ·0015CO_2	
			42·9° C.	.	. ·0014CO_2	

Also it was found [†] that the leaves of this plant in their respiration exhibit the same relation to temperature as they do in their carbon assimilation in that the initial value at higher temperatures is not maintained but falls away, the higher the temperature the steeper the fall.

An increased temperature must be operative for some time before the stimulation of the respiratory process is noticeable, a sharp variation in temperature is without effect and a change in the respiration intensity after transference from one temperature to another is gradual.[‡]

Müller-Thurgau and Schneider-Orelli [§] in their investigation on the respiration of potatoes found much the same phenomenon to obtain : mere variation in temperature effected by heating and cooling is without effect unless the exposure to the higher temperature be sufficiently prolonged. Potato

[*] Matthæi: "Phil. Trans. Roy. Soc." Lond., B., 1904, **197**, 47.
[†] See Blackman : "Ann. Bot.," 1905, **19**, 281.
[‡] Blanc : " Rev. gén. Bot.," 1916, **28**, 65.
[§] Müller-Thurgau and Schneider-Orelli : " Flora," 1910, **101**, 309.

tubers, after an exposure to a temperature of 40° C. for several hours and then transferred to room temperature, show a gradual increase in respiration intensity which reaches its maximum after a lapse of twenty-four hours. Exposure to higher temperatures, e.g. 44° C., results in a permanent increase in the intensity of respiration which indicates a permanent change in the organism. To obtain this stimulation the temperature must be sufficiently high, no effect being observed for temperatures below 38° C. This is the minimal temperature for the phenomenon described in the potato, but the value probably varies in different plants. In the instance of the potato, the difference between the maximum intensity of respiration of heated tubers and that of unheated tubers is proportional to the difference between the minimal temperature and the increased temperature to which the tubers were exposed. Thus it was observed that after exposure to 44° C. the respiration was two and a third times as great as after an exposure to 40° C. These authors consider that the effect of the high temperature is adversely to affect the protoplasm in much the same way as does old age : as a result of this decadence, less starch is formed by the leucoplasts ; also the enzymes are less active so that there is less invert sugar for the leucoplasts to fix. For instance, at 18° C. previously heated potatoes contain a relatively large amount of sugar ; at 0° C., which temperature inhibits starch formation in normal tubers,* the much slower formation of sugar indicates that the increased temperature also has had an injurious effect on sugar formation although the amount of diastase apparently is unaltered. The weakening effect of high temperature also is seen in the respiratory activity of wounded potatoes ; the general effect of wounding (see p. 78) is to increase the intensity of respiration, which increase is maintained. After wounding there is no immediate respiratory response in previously heated tubers and even when recovery from the heating effect had apparently been made the maximum intensity of respiration due to the traumatic stimulus never reached so high a degree as in unheated wounded tubers.

Dormant structures were the subjects of this investigation ; the subjects for the work of Kuijper,† on the other hand, were

---

* See Vol. I., p. 132.
† Kuijper : " Extr. Trav. Bot. Néerland.," 1910, **7,** 130.

active seedlings of the lupin, pea and wheat. The seeds were germinated at about 20° C. and then exposed to various temperatures, the carbon dioxide being estimated hourly for six
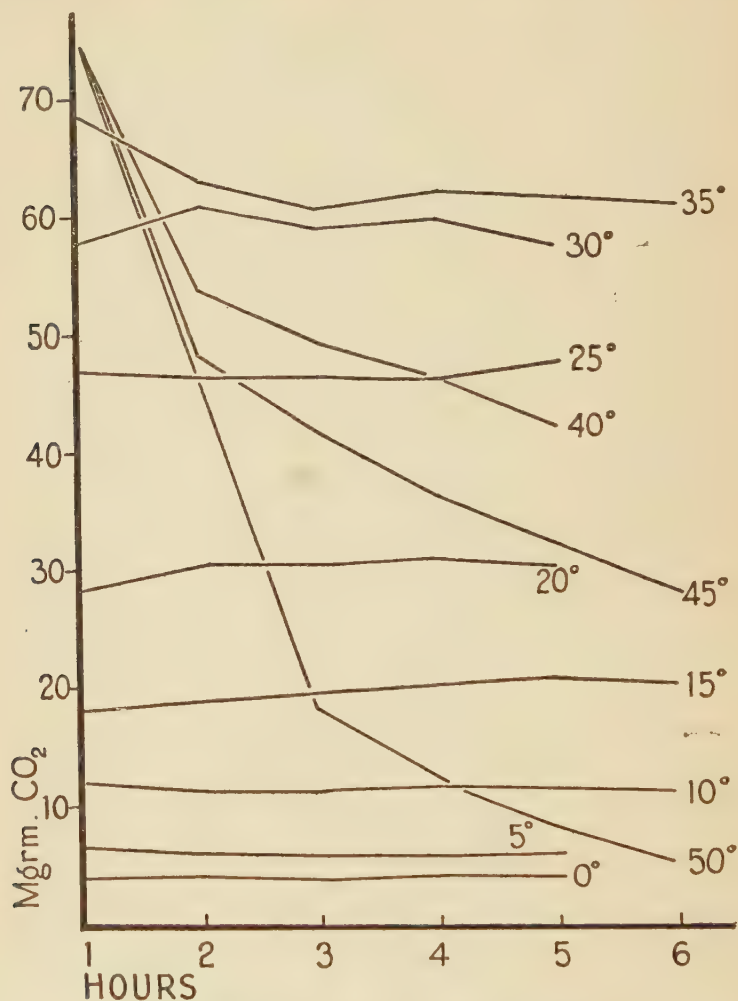
FIG. 6.—Respiration of the pea at various temperatures.

hours (Fig. 6). At temperatures not exceeding 10° C. the output of carbon dioxide is constant; from 10° C. to 20° C. there is a continuous rise in the respiration of seedlings of all ages, which rise is followed by a fall; at temperatures above 20° C. and up

to 40° C. the amount of carbon dioxide evolved fluctuates: a rapid fall occurs in the first two hours, then a rise for one or two hours, followed by a continuous fall. At still higher temperatures there is a continuous reduction in the evolution of carbon dioxide, a result which is in accordance with the observations of Blackman mentioned above.

These observations are different to those made by Müller-Thurgau and Schneider-Orelli; thus Kuijper observed a permanent increase in respiration after due exposure to high temperatures, whilst the latter workers found a decrease to obtain. This discrepancy may be due to the fact that Kuijper's seedlings were subjected to the increased temperature for a lesser time than the potatoes of Müller-Thurgau and his collaborator and, as has already been remarked, the material employed was in active growth on the one hand whilst on the other it was resting. Kuijper explains his results on the hypothesis that there are two distinct processes concerned in respiration which are affected differently by a continuance of high temperature: one phase being early depressed whilst the other is stimulated; there is here a possible correlation with the dual origin of the carbon dioxide of respiration, which, according to Palladin, arise from the activity of an oxidase and of a carboxylase. Like Müller-Thurgau and Schneider-Orelli, Kuijper recognizes the inter-relation of temperature and the nature of the available respirable material. Thus of the seedlings examined, the lupin showed a rise in the respiration intensity from 15° C. to 20° C., the pea from 20° C. to 25° C., and the wheat at 30° C. The fluctuation period was well marked at 25° C., 30° C., and 35° C. respectively and the continual fall in carbon dioxide output obtained at 25° C., 35° C. and 40° C. respectively. Of the food available, the lupin has 37 per cent, the pea 22 per cent, and the wheat 12 per cent of protein, whilst of starch the lupin has none, the pea 54 per cent, and the wheat 74 per cent.

If respiration be a purely chemical process, a combustion of, say, sugar, Van't Hoff's law should apply throughout the process: the evidence shows, however, that the law is followed only for lower temperatures; at higher temperatures, as has been seen, fluctuations obtain and correlation comes to an end, which is indicative of the intervention of internal factors, the

nature of which is not clearly understood. Clausen * in the
case of seedlings and buds found the rate of evolution of carbon
dioxide to be about doubled with an increase of 10° C., and
Kuijper found the law to apply at temperatures between
0° C. and 20° C. in the pea and wheat, and between 0° C.
and 25° C. for the lupin, the coefficient lying between 2 and
3 for a difference in temperature of 10° C.

With regard to low temperatures, it is a well-known fact
that seeds, lichens, mosses, etc., especially when dry, retain
their vitality under exposure to intense cold ; even so low a
temperature as – 250° C. will not cause death. Since respira-
tion is in its complete expression a concomitant of life, the
process must continue in an attenuated form even at so low
a temperature as that of liquid air.

FOOD.—The continuance of respiration ultimately depends
upon adequate food supplies, and the intensity of respiration
may be much influenced by the presence of substances
immediately available as respirable material. Formerly it was
considered that sugar, more especially dextrose, was essentially
the respirable material, but no statement of general applica-
tion can be made. The action of foods in this connexion
varies with different plants and their physiological condition,
and is influenced by the operation of various natural internal
factors, such as acidity, which are not directly controllable in
experimental work.

But notwithstanding this, general physiological experience
shows that carbohydrates are highly important in aerobic
respiration. Palladin,† for instance, found that 100 grams of
etiolated bean leaves, with depleted carbohydrate, gave off
102·8, 95·9 and 70·2 mg. of carbon dioxide respectively
for three successive hours ; but after these leaves had been
grown in darkness on a solution of cane sugar for two days, by
which means their carbohydrate content was increased, the
rate of evolution of carbon dioxide was 152·6, 147·5, 146·8,
and 144·5 mgs. respectively for four successive hours.

A more prolonged culture on cane sugar does not result in a
corresponding increase in the rate of carbon dioxide produc-

* Clausen : " Landw. Jahrb.," 1890, 19, 893.
† Palladin ; " Rev, gén. Bot.," 1893, 5, 449.

tion although the carbohydrate reserve is greater ; thus after
a treatment over a period of forty hours, the leaves evolved
no more carbon dioxide than they did after four hours. Such
observations indicate that a certain content of carbohydrate is
necessary, but an increase above this value, which is a vari-
able figure, is merely increasing the respirable capital of the
organ.

Müller-Thurgau and Schneider-Orelli * found that the
respiration of sweet potatoes is very high compared with
normal tubers ; in the autumn, when little if any sugar is
present, the respiration is low, but as the tubers increase in age
and sugar accumulates there is concurrently a more intense
respiration. According to Knudston † the respiration of the
vetch is intensified to different degrees by saccharose, glucose
and maltose, the last mentioned being less effective than the
others. Spoehr,‡ from his investigations on the carbohydrate
economy of the Cactaceæ, concludes that the rate of respira-
tion is not controlled by any one group of sugars, thus hexose
sugars, which often are considered to be of exceptional im-
portance in respiration, occur in the Cactaceæ in variable
amounts under varying conditions of existence and sometimes
may be almost absent, but the respiration intensity is not
thereby reduced in any marked degree. Under conditions
which involve a poor supply of hexose sugars, the polysac-
charides are consumed in the respiratory processes and such con-
ditions are possibly connected with the formation of pentosanes.
The conditions referred to are temperature and water : a low
water content accompanied by a high temperature bring about
a decrease of monosaccharides and an increase of polysac-
charides and of pentosanes, whilst the contrary conditions, a
high water content and low temperature, are associated with
an increase of monosaccharides and a decrease of polysac-
charides and of pentosanes.

With regard to the proteins, Palladin § has shown that
during the germination of wheat in darkness the total protein
content and the soluble carbohydrates diminish whilst the

* Müller-Thurgau and Schneider-Orelli, loc. cit.
† Knudston : " Cornell Agric. Exp. Sta. Mem.," 1916, 9, 1.
‡ Spoehr : " Carnegie Inst. Pub.," 1919, No. 287.
§ Palladin : " Rev. gén. Bot.," 1896, 8, 225.

evolution of carbon dioxide and proteins insoluble * in pepsin, increase in amount. As development proceeds, the carbohydrates, which originally were abundant, become depleted and the decomposition of protein is less vigorous and may even cease. Hence in the later stages of germination there is a more vigorous respiration under conditions of low protein content than in the early phases of germination when more protein obtains. The proteins which disappear are those of the reserve food, not those which are presumably members of the protoplasmic complex and which show a direct relation to the amount of carbon dioxide evolved. This is shown in the following table which relates to wheat germinating at 20° to 21° C. and gives the ratio of the carbon dioxide evolved per hour to the amount of protein unacted upon by pepsin :—

Age of Seedling in Days.	$\frac{CO_2}{N_2}$.
4	1·06
6	1·05
7	1·18
9	1·15

Palladin found a similar ratio to obtain in other plants, the following being some of the values obtained :—

Wheat seedlings .	1·05, 1·15, 1·07, 1·18
Lupin seedlings .	1·12
Etiolated leaves of the broad bean after cultivation on sugar solution .	1·10

The differences between these observed ratios are not considered to be significant by Palladin in view of the experimental difficulties. From his observations he draws the general conclusion that for a given temperature and with an adequate amount of carbohydrate, the relation between the quantity of carbon dioxide evolved by different plants in one hour and the quantity of protein unacted upon by gastric secretion, *in vitro*, is a constant. For a temperature of 19° to 20° C., $CO_2/N_2 = 1·1$.

The amount of the protein referred to, as Kidd, West and Briggs have pointed out, is a measure of the amount of respiring cell matter.

With regard to the fats, there is but little precise information available from the point of view of respiration. Their conversion to carbohydrate is one phase of

* The solubility being determined *in vitro*.

the involved problems, and necessitates a much greater up-take of oxygen in those instances where fats are the pre-dominating respirable material as compared with those in which carbohydrate is the prime respirable substance, that is, the respiratory quotient of a fat-containing seed is of a com-paratively low value. To what extent the products of the hydrolysis of fats are immediately respirable, is another aspect of the subject awaiting investigation.

WATER.—Since an ordinary living plant is mostly water, it is obvious that any circumstance which leads to a marked increase or decrease of water may alter profoundly the ex-pressions of life ; thus if water be removed from a plant to an extent sufficient to make the cells flaccid, growth will come to an end and therefore the respiratory activity will be ultimately considerably lessened. This statement is intention-ally qualified, for an increase and also a decrease in the water supply may lead to an immediate amplification in the intensity of respiration. Maige and Nicolas * found in several instances that a rise in turgescence is followed by an increase in respiratory activity and a similar result obtains, although to a lesser degree, when a decrease in turgescence is brought about in similar material either by natural evaporation or by the action of a mildly plasmolyzing sugar solution. If a further removal of water be effected by the use of a 10 to 20 per cent solution of glucose, a decrease in respiration, as indicated by a smaller intake of oxygen and output of carbon dioxide, results. Smith † similarly observed that vegetative organs of the snowdrop, the bean, and *Tropæolum*, after drying in a desiccator until a third to a half of the water had been removed, showed a greater respiration as compared with normal members of the plants mentioned. Up to a loss of 30 per cent of water the respiratory activity increased in pro-portion to the amount of water lost ; from 30 to 60 per cent loss of water the respiration was uniform at the increased rate attained with the 30 per cent loss ; finally, with a loss of from 60 to 100 per cent of water the respiration decreased propor-tionately to the amount of water removed. All plants do not

* Maige and Nicolas : " Rev. gén. Bot.," 1910, 22, 409.
† Smith : " British Ass. Rep.," 1916, 85, 725.

behave alike in these respects ; the paeony and the asparagus show no increase in respiration following removal of water, and Palladin and Sheloumova * found that potato tubers exhibited a lowering of respiration on dehydration under the conditions of their experiments which were not the parallels of those of the authors just quoted.

There is no agreed explanation of these facts : Smith suggests that the phenomenon is due to changes effected in the activity of the enzymes involved, whilst Maige and Nicolas consider that the increased turgescence increases growth and consequently respiration whilst the decreased turgescence stimulates respiration since the respirable materials are concentrated. If this be so, it is reasonable to suppose that there is, *mutatis mutandis*, an optimal concentration of these substances and it is the passing of this strength which accounts for the diminution in respiration in tissues subjected to the plasmolyzing action of strong sugar solutions.

The long periods of time through which certain plants can sustain life on a diminished water supply is remarkable : Long † found that a shoot of *Echinocactus* retained life and exhibited respiration after a period of desiccation of thirty months, the first eight in full sunshine and the remainder in a dark room at air temperature. Seeds, however, provide the most striking instances and the importance of a knowledge of the conditions affecting respiration, and consequent heating, of stored seeds is great. It is not unlikely that the respiration of really dry seeds is, to a large extent, anaerobic ; in air-dry seeds there is a small evolution of carbon dioxide which may have its origin in the testa only.‡ With regard to the respiration of bulked grain reference may be made to the results obtained by Bailey and Gurjar.§ These authors find that the spontaneous heating of damp grain is chiefly due to the activity of the embryo in oxidizing sugars, the degree of respiration increasing with the amount of available water. Up to 14·5 per cent of water there is a uniform and gradual rise in the intensity of respiration ; in greater proportions, water will

* Palladin and Sheloumova : " Bull. Acad. Sci. Petrograd," 1918, 801.
† Long : " Bot. Gaz.," 1918, 65, 354.
‡ Becquerel : " Ann. Sci. Nat. Bot.," 1907, 5, 193.
§ Bailey and Gurjar : " Journ. Agric. Res,," 1918, 12, 685.

produce a marked respiratory acceleration. In this respect glutenous material * is important in that its degree of viscosity is lowered as it takes up water; this permits a more rapid diffusion and hence a greater respiration. This is illustrated in the fact that soft starchy varieties of wheat exhibit a higher respiration intensity than hard vitreous wheats with the same percentage of water. The embryo is the seat of enzyme secretion; shrivelled wheat, which contains less endosperm and the same amount of embryo as compared with plump wheat, shows a respiratory activity twice or thrice as much as plump with the same water content, 14 per cent; with less water than 14 per cent, there is little or no difference between the two kinds of grain. The effect of respiration in bulked grain is a rise in temperature; this is cumulative and causes an acceleration in respiration up to about 55° C., which temperature also has the effect of stimulating diastatic activity and hence rendering available more immediately respirable material. On the other hand, the resulting accumulation of carbon dioxide has a narcotic effect and reduces the rate of respiration.

SALTS.—The action of salts on the intensity of respiration varies according to their chemical nature, their concentration and their association with other salts; thus salts of the heavy metals are very toxic whilst those of the alkali metals and alkaline earths accelerate or retard according to their concentration and association. *Nitrosomonas* shows a maximum respiration in $N/200$ ammonia whilst a strength of $N/10$ depresses respiration in a marked degree.† Similarly the respiration of *Bacillus subtilis* is constant in the presence of low concentrations of the chlorides of sodium, potassium and calcium; an increase in concentration leads to a reduction in respiration. As compared with cultures lacking these salts, the respiration intensity is increased by the presence of sodium chloride, potassium chloride, calcium chloride or magnesium chloride in concentrations of $\cdot 2M$, $\cdot 15M$, $\cdot 05M$, and $\cdot 03M$ respectively. In higher concentrations the rate of respiration is decreased.‡ The use of mixtures of salts accelerate or retard

* Vol. I., p. 333.
† Meyerhof: "Pfluger's Archiv.," 1917, 166, 240.
‡ Brooks: "Journ, Gen. Physiol.," 1919, 2, 5; 1920, 2, 331.

according to the degree of their antagonism : thus the normal respiration rate of *Aspergillus niger* in a culture medium containing 19 c.c. of ·5M sodium chloride and 1 c.c. of ·5M calcium chloride is maintained although each of these salts in these concentrations and acting alone will accelerate respiration.* With regard to respiration, the chlorides of magnesium and sodium, of sodium or potassium and calcium exhibit a conspicuous antagonism, whilst the chlorides of magnesium and calcium and of sodium and potassium are antagonistic only to a slight degree.

ACIDITY.—The degree of acidity of the cell sap shows much variation and is an expression of the physiological condition and of particular metabolism. Thus the acidity of anthocyanin containing leaves may be double that of green leaves of the same species, the acidity of fleshy plants is greater by night than by day.

In particular cases the hydrogen ion concentration may profoundly modify the respiration intensity ; *Nitrosomonas*, for example, shows the greatest rate of respiration in a medium in which the P_H value is between 8·4 and 8·8, beyond the limits of P_H 9·4 – 7·6 the process comes to a standstill.† In *Penicillium chrysogenum* variations in the value of P_H between 4 and 8 do not affect the normal rate of respiration, that is, the rate at neutrality, $P_H = 7$; an increase of the value to 8·8 results in the respiration decreasing to 60 per cent of the normal, at which level it remains ; a decrease, on the other hand, in the value of P_H to 2·65 causes a gradual rise in the respiration rate followed by a gradual fall to the normal. At P_H 1·10 to 1·95 the preliminary rise, amounting to 20 per cent, is followed by a fall to below normal. The depression brought about by a concentration $P_H = 1·95$ or less is irreversible, whilst the similar decrease effected by a P_H value of 8·8 is reversible, the respiration rate returning to normal after the plant is placed in a neutral solution. In acid solutions there is an increase in the production of carbon dioxide and a decrease in alkaline solutions, a phenomenon which may be paralleled in the test-tube : a neutral solution of dextrose and

* Gustavson : " Journ. Gen. Physiol.," 1919, 2, 217.
† Meyerhof : loc. cit.

hydrogen peroxide show an increase in the evolution of carbon dioxide on the addition of acid but not on the addition of alkali.* Witzemann † also has demonstrated the oxidation of sugar by hydrogen peroxide in the presence of disodium hydrogen phosphate. Whether the phosphate here plays any part in the formation of a hexose phosphate such as is known to occur in alcoholic fermentation ‡ has yet to be demonstrated.

LIGHT.—That plants respire both by day and by night is a well-known fact from which it would appear that radiant energy as such is not a conditioning factor in respiratory activity; its action is indirect in providing through its photosynthetic activity a supply of respirable material. Thus an isolated green leaf in darkness shows a continuous fall in respiration; in the light, on the other hand, the respiratory values during a carbon assimilation experiment are continually changing by virtue of the carbon assimilation and may be doubled by an exposure of four or five hours to light and carbon dioxide. This is possibly due to the production of new carbohydrate although the observed increase is not proportional to the amount of carbon dioxide decomposed.§ The high intensity of respiration sometimes observed in green plants in bright sunshine ‖ is due, at any rate in part, to the copious supply of sugar. Under such conditions, however, other factors are operative : the higher temperature, for instance, would increase the respiration intensity ; whilst if the illumination, the temperature and humidity conditions of the atmosphere were operative all in the same direction to cause undue loss of water, the flaccid leaves would ultimately show a respiratory depression.

In addition to this indirect action of light, there appears to be some direct action, for Spoehr ¶ finds that the respiration of the plant is regularly higher under conditions which vary only as regards daytime air and night-time air. The differences observed are, however, not considerable ; in the case of

* Gustavson : " Journ. Gen. Physiol.," 1920, **2**, 617 ; **3**, 35.
† Witzemann : " Journ. Biol. Chem.," 1920, **45**, 1.
‡ Vol. I., p. 379.   § Matthaei : loc. cit.
‖ See Rosé : " Rev. gén. Bot.," 1910, **22**, 385.
¶ Spoehr : " Bot. Gaz.," 1915, **59**, 366.

the wheat the ratio day rate/night rate was found to be 1·042 in normal air and 1·010 in deionized air. The reason for the difference is not obvious : it is known that during daylight the air becomes ionized to various degrees under conditions of low relative humidity. Ionized oxygen is more potent than deionized oxygen so that the ionized oxygen of the daytime air, according to Spoehr, possibly accelerates the purely oxidative process of respiration, not the initial disruption of the respirable material. But until it can be said with certainty at what stage in respiration oxygen becomes operative, an adequate explanation is not possible.

THE MECHANISM OF RESPIRATION.

The catabolic processes of plants may be directly referable to specific enzymes, zymase for instance in alcoholic fermentation ; but in the respiratory activities of higher plants, their rôle is not defined with that precision and degree of completeness which is desirable, although it is generally agreed that enzyme action plays an important part in the process. Enzymes associated with the common end products of respiration are subjects for first consideration and of them most attention has been given to oxidase, peroxidase, catalase, zymase, and carboxylase.

Oxidase, to use the generic term, has remarkable properties of effecting with rapidity the oxidation of various substances in the presence of oxygen.[*] They are of wide occurrence in the vegetable kingdom, as should be the case if they are primarily concerned in aerobic respiration, but whether they are present in all living cells is doubtful. According to Atkins [†] they are absent or inactive in tissues markedly acid in reaction or containing large amounts of reducing substances. Bunzel [‡] also has shown that the activity of these enzymes is inhibited by acids, their greatest activity being at or near the point of neutrality ; the limits of the P_H value corresponding to complete inhibition in the various subjects of experiment are narrow and the figure of acid sensitiveness is almost invariable in a particular genus.

[*] See Vol. I., p. 392.
[†] Atkins : " Proc. Roy. Dublin Soc.," 1913, 14, 144.
[‡] Bunzel : " Journ. Biol. Chem.," 1916, 28, 153.

In view of the importance ascribed to this class of enzyme in respiration processes, it is desirable here to reconsider them.* A freshly prepared 1 per cent alcoholic solution of guaiacum resin, made by dissolving pieces of the resin from which the outer layer has been scraped off, forms a delicate reagent for a certain type of oxidizing system which turns the solution blue on being brought into contact. When properly prepared, the guaiacum solution is not turned blue on exposure to the air, nor by the addition of hydrogen peroxide. If, however, a mixture of guaiacum and hydrogen peroxide be brought into contact with the juice of certain plant tissues, either the expressed sap or a freshly-cut surface, such as a slice of horse radish, a blue colour is immediately assumed. From this it may be concluded that the tissue contains a material capable of setting in motion some oxidizing mechanism which causes the oxidation of guaiacum to the blue compound. Horse radish and like substances exhibiting a similar action are said to contain a peroxidase, that is, an enzyme which can activate hydrogen peroxide so as to make it turn guaiacum blue. Peroxidase is, in fact, assumed to act in much the same way as does ferrous sulphate upon a mixture of hydrogen peroxide and tartaric acid : if a little hydrogen peroxide be added to a solution of tartaric acid, no change is observed ; on the addition of a few drops of ferrous sulphate, however, a yellow coloration is immediately produced which changes to violet on the addition of caustic soda. The ferrous sulphate activates the hydrogen peroxide to oxidize the tartaric acid to dihydroxymaleic acid. Two kinds of oxidizing enzymes are now generally recognized in the plant world, namely, the direct acting oxidases which turn blue an alcoholic solution of guaiacum without the addition of hydrogen peroxide, and the indirect acting peroxidases which are unable to produce a change in colour until hydrogen peroxide has been added. To account for these facts, it was assumed that whereas the peroxidase required the addition of a peroxide in order that it might have something from which to liberate active oxygen, the oxidase contained not only the peroxidase but also a

* See Vol. I., p. 392.

second enzyme, an oxygenase,* whose function was to generate with the help of atmospheric oxygen the requisite peroxide. The oxidase system therefore was direct acting owing to the fact that it contained the necessary mechanism in the oxygenase for generating its own peroxide and so making it independent of any added hydrogen peroxide. This view was supported by the fact that by the fractional precipitation with alcohol of an aqueous extract of *Lactarius*, the contained oxidase could be separated into its two constituents :—

(*a*) A portion soluble in alcohol, the peroxidase constituent, which was without direct action on guaiacum.

(*b*) A portion insoluble in alcohol, the oxygenase constituent, which acted but feebly upon guaiacum, but showed a much stronger action on the addition of the alcohol soluble fraction.

The presence of an oxidase in a plant tissue may also be revealed by bruising the surface or by exposing to chloroform vapour. Under such treatment the cell contents are somehow disturbed, with the result that the oxidase contained therein causes the formation of a coloured substance in the substrate.

The observation of Wheldale Onslow † that the oxidase contained in the pea and in the potato are able to oxidize pyrocatechol and allied substances,‡ suggest that the browning on injury is due to the action of an oxidizing enzyme upon some such substance contained in the tissue injured. In support of this view, the presence of a substance of a pyrocatechol nature may be demonstrated as follows. By grinding thin slices of potato under 96 per cent alcohol, the oxidase system may be divided into its two constituents, an alcohol soluble portion and the peroxidase which owing to

* Bach and Chodat : "Ber. deut. chem. Gesells.," 1903, **36**, 606; 1904, **37**, 36, 1312.

† Wheldale Onslow : "Biochem. Journ.," 1919, **13**, 1.

‡ The substances to which reference is made are protocatechuic and caffeic acids.

Pyrocatechol.   Protocatechuic acid.   Caffeic acid.
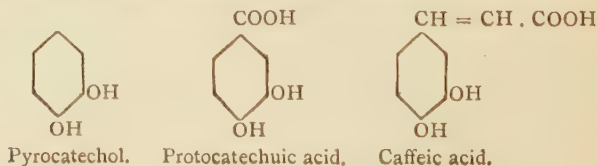
its insolubility in alcohol is precipitated on the potato residue. This potato residue no longer turns blue guaiacum directly, but does so on the addition of hydrogen peroxide. In order to demonstrate the presence of the pyrocatechol grouping in the alcohol soluble extract, the following procedure may be adopted. Boil 500 grams of slices of freshly-peeled potato for a quarter of an hour in 250 c.c. of 96 per cent alcohol over a water bath. After filtering, evaporate off the alcohol and extract the residue with a little warm water and filter. To the filtrate add a concentrated solution of lead acetate until no further precipitate is formed. Filter off the precipitate and after washing, suspend it in a little water and add 10 per cent sulphuric acid drop by drop until the yellow colour of the precipitate turns white owing to the formation of lead sulphate. Filter and carefully neutralize the filtrate with 1 per cent caustic soda. The presence of the pyrocatechol grouping may be demonstrated by the addition of a drop of ferric chloride which produces a green colour ; on adding a few drops of 1 per cent sodium carbonate, the colour changes to blue and then to reddish purple. According to Wheldale Onslow it is the presence of this pyrocatechol group in the substrate which provides the peroxide for the peroxidase to activate and there is consequently no need to postulate the existence of a second enzyme or oxygenase as is assumed by Bach and Chodat. The existence of an oxygenase also is disputed by Moore and Whitley.*

In addition to guaiacum, other reagents have been used in the investigation of oxidizing enzymes ; amongst these may be mentioned benzidene, a-naphthol, p-phenylene diamine, and pyrogallol ; none of these, however, produce any colour with plant tissues or extracts unless hydrogen peroxide be added. This is a very significant fact, inasmuch as all oxidizing enzymes found in plants would appear to be peroxidases as tested by these reagents and the distinction between oxidase and peroxidase would therefore appear to be dependent upon the reagent employed. Contrariwise the discovery is within the bounds of possibility of a reagent of sufficient delicacy to be changed in colour by direct action not only by plant

* Moore and Whitley: "Biochem. Journ.," 1909, 4, 136.

material containing so-called oxidases but also by material which at present only gives the indirect action. If this substance were known, the distinction between peroxidase and oxidase would no longer be necessary, as indeed it would seem even now to be, inasmuch as this distinction is entirely dependent upon the external reagent, guaiacum, employed and disappears when another reagent is used. Moreover, if in order to activate a peroxidase it is necessary to add hydrogen peroxide, since the plant itself does not supply the necessary peroxide, unanswerable questions arise regarding the employment of these enzymes in those plants which contain only peroxidases.

It would, in fact, appear to be more likely that the difference between oxidase and peroxidase is one of degree rather than of kind, and that the so-called peroxidase is really only a slightly less powerful oxidizing enzyme than the so-called oxidase; the lack in power may be due to a lower concentration, as has already been suggested by Ewart,[*] or to some slight difference in the molecular complex, rather than to a different mechanism involving the necessity for the presence of a third substance.

An alternative explanation of the working of oxidizing enzymes has been formulated by Wieland and has been considered in previous pages.

Catalase, which has the property of setting free gaseous oxygen from hydrogen peroxide, although of common occurrence, is not universally present in living cells.

Zymase is the enzyme particularly associated with yeast and is responsible for the alcoholic fermentation of sugar. It has been described as occurring in higher plants such as the beetroot, potato, lupin, and others.[†]

Carboxylase has the property of removing carbon dioxide from carboxyl groups, thereby converting pyruvic acid, for example, into carbon dioxide and acetic aldehyde;[‡] it occurs in yeast, where it is associated with zymase, and, according to Bodnar,[§] in the beetroot and potato tuber.

[*] Ewart : " British Ass. Rep.," 1915.
[†] Palladin and Kostytschev: " Ber. deut. bot. Gesells.," 1906, **24**, 273; Stoklasa and Chocensky : *Id.*, 1907, **25**, 122.
[‡] See Vol. I., p. 383.
[§] Bodnar : " Biochem. Zeitsch.," 1916, **73**, 193 ; see also Zaleski : " Ber. deut. bot. Gesells.," 1913, **31**, 349.

If respiratory processes are esentially enzymatic, it is natural to suppose that the respiration intensity would increase in the presence of accelerators : various substances have been so described, amongst them being phosphates * which are important accelerators in zymase action. †

Palladin looks upon lipoids as being of the nature of co-enzymes, since the more they are extracted from the plant tissue, the greater is the reduction in the intensity of respiration although not in an exact proportion.‡

Galitsky and Wassiljeff § observed that the addition of boiled water extracts of bean seeds and wheat grains increased the output of carbon dioxide in living and in dead seedlings to a degree depending on the acidity of the culture medium : in neutral cultures the increase reached 117 per cent, whilst in a slightly alkaline medium an increase of 86 per cent obtained as compared with an increase of 60 per cent in a slightly acid medium.

Of the enzymes mentioned, oxidase and catalase are the more prominent, numerous observations having been made on their distribution and relation to the oxidative aspects of respiration. Appleman ‖ found that the oxidase content of the expressed juice of the potato is not indicative of the intensity of the respiration of the tuber, whilst the catalase activity shows a striking correlation. Similarly for sweet corn, in which instance the respiration in the milk stage is high when first collected but in storage rapidly decreases, the decrease being accompanied by a nearly proportional fall in catalase activity. In the pine-apple, Reed ¶ found that oxidase and catalase are independent ; the amount of the former remains constant during ripening of the fruit whilst the catalase increases. In the wheat and certain allied plants, the embryo shows a twenty-eight to twenty-nine-fold greater catalase and oxidase activity than the endosperm, and this also holds for the intensity of respiration. In air-dry fruits of *Andropogon*

* Iwanoff : " Biochem. Zeitsch.," 1910, **25**, 171 ; 1911, **32**, 74 ; Zaleski and Reinhard : *id.*, 1910, **27**, 450.

† See Vol. I., p. 379.

‡ Palladin : " Ber. deut. bot. Gesells.," 1910, **28**, 120.

§ Galitzky and Wassiljeff : *Id.*, 182.

‖ Appleman : " Amer. Journ. Bot.," 1916, **3**, 223 ; 1918, **5**, 207.

¶ Reed : " Bot. Gaz.," 1916, **62**, 409.

halepensis, catalase activity runs parallel to respiration, a corre-
lation which does not obtain in the seeds of *Amaranthus.**   In
Acer saccharinum, Jones † found that the intensity of respiration
of seeds during the process of desiccation at 25° C. at first de-
creases, then rises to a maximum and finally a gradual decline
to zero : with regard to the catalase activity, there is a slight
initial increase, then a decrease as desiccation proceeds, during
which process there is also a gradual decrease in peroxidase
activity.   In the early stages of germination, when respiration
is high, there is a large increase in catalase activity.   From
these results it would appear that catalase and respiration are
more closely correlated than are oxidase and respiration.   It
is to be remembered, however, that catalase and oxidase are
closely related enzymes and that agreement has not been
reached regarding the interrelation of these enzymes in the
oxidizing complex, an aspect of the subject which has before
been considered.‡

There appears to be no general concurrence of opinion re-
garding the precise mode of action of these respiratory
enzymes.   Palladin is one of the few who have formulated a
more or less complete scheme of the mechanism, but before
considering his thesis, it is desirable briefly to draw attention
to the salient phenomena presented in the anaerobic respira-
tion of higher plants.   It has been mentioned that the higher
plants, although normally aerobic, may be facultatively anaerobic :
the anaerobic respiration of germinating pea seeds, for example,
is a commonplace of the laboratory.   In such instances the car-
bon dioxide evolved certainly has no relation to initial gaseous
oxygen ; it represents a dissociation product of the substances
physiologically consumed.   Comparison with the alcoholic fer-
mentation of sugar is an obvious pursuit and the two phe-
nomena show remarkable resemblances.   Anaerobic respiration
is an extravagant method of obtaining energy and gives rise to
carbon dioxide and ethyl alcohol which in a sense is wasted un-
less further oxidized.§   The amount of alcohol and carbon
dioxide produced is a variable quantity depending on the

* Crocker and Harrison : " Journ. Agric. Res.," 1918, **15**, 137.
† Jones : " Bot. Gaz.," 1919, **69**, 127.
‡ Vol. I., p. 396.
§ See Kostytschev : " Journ. Russ. Bot. Soc.," 1916, **1**, 182.

amount of carbohydrate available for reduction in the plant and on the experimental conditions employed.*

Thus the amount of carbon dioxide evolved from etiolated bean leaves, in an atmosphere free from oxygen, is negligible, but if kept with their petioles immersed in a solution of sugar for some time previous to their being placed under anaerobic conditions much carbon dioxide is produced and their life is more prolonged. With regard to alcohol, a similar correlation obtains; in a specific instance etiolated bean leaves under anaerobic conditions gave 256·8 mgs. carbon dioxide and 68·3 mgs. of alcohol in thirty hours, a ratio of 100 : 26·5, whereas leaves previously given sugar yielded under precisely similar conditions 782·4 mgs. of carbon dioxide and 724·6 mgs. of alcohol a ratio of 110 : 92·6.†

Whether zymase and carboxylase are of general occurrence in all those normally aerobic plants or members of plants which are capable of living for a time under anaerobic conditions remains to be discovered.

This survey, brief though it be, is sufficient to indicate the close connexion between, if not the identity of, anaerobic respiration and fermentation. Amongst the lower plants studied, this parallelism is not so obvious, thus Kostytschev ‡ found that mushrooms containing no sugar § give origin to much carbon dioxide but no alcohol when grown under anaerobic conditions, possibly because the alcohol is oxidized almost as soon as it is formed: but however this may be, more information is necessary before an adequate attempt can be made to correlate the catabolic processes of these and like plants possessed of such plastic metabolic methods.

Reference may now be made to Palladin's ideas on respiratory processes. With regard to the origin of carbon dioxide, he considers that there are three possible sources: that arising from the activity of enzymes more or less closely associated

* Palladin: "Rev. gén. Bot.," 1894, 6, 201.

† Palladin and Kostytschev: "Abderhalden's Handbuch," 1910, 3, 479.

‡ Kostytschev: "Ber. deut. bot. Gesells.," 1908, 25, 188; 26a, 1674; "Zeit. physiol. Chem.," 1910, 65, 350.

§ Glucose has been described as occurring in the mushroom and allied plants. The amount varies greatly, according to the conditions under which the plants are grown, and varies, not only in different batches, but also in individual plants of one batch. These fungi also contain carbohydrates capable of yielding sugars.

with the protoplasm and which may or may not be found in the expressed sap ; that produced directly from the protoplasm under stimulation such as wounding, which aspect of the subject has already been considered ; and that produced as a result of oxidase activity.*   With respect to the production of carbon dioxide by oxidase enzymes, Palladin accepts Bach and Chodat's hypothesis regarding the constitution of these enzymes and considers that this phase in respiration depends on the presence of an oxidizable substance which under the action of the oxygenase yields an organic peroxide which transfers oxygen, when stimulated by the peroxidase.

Palladin further accepts Bach and Chodat's opinion that oxygenases, if they exist, are very unstable and are quickly used up ; it is for this reason that the presence of oxygenases is difficult to demonstrate.   This idea of the action of oxidases in respiration is bound up with the tenability of Bach and Chodat's opinion regarding these bodies.   There is, however, much to be said for the thesis that respiration is not necessarily one single operation but consists of at least two, of which one is the oxidation of substances by enzymes only indirectly related to the protoplasm and is not therefore a concomitant of life.   Palladin and Kostytschev, for instance, showed that germinated peas which had been killed without injury to the enzymes may give off more carbon dioxide than during life, and so also may the bulb of an onion, killed by exposure to a temperature of $-20°$ C. on thawing, although in this instance the amount of oxygen absorbed is less than in the living condition.   Haas † found that plants of *Laminaria* poisoned by various substances such as ethyl bromide, acetone, and alcohol first showed a rise in respiration followed by a smooth decline to zero ; at the death point the evolution of carbon dioxide was not markedly smaller, and it may be considerably greater, than the normal rate in living tissue.   But this post mortem respiration is not shown in all instances : Palladin observed that the evolution of carbon dioxide from finely ground wheat is less than from the living intact grains.

He also made a comparative study of the effects of various poisons on the evolution of carbon dioxide from living and

* Palladin : " Ber. deut. bot. Gesells.," 1905, **23**, 240 ; 1906, **24**, 97.
† Haas : " Bot. Gas.," 1919, **67**, 347.

dead tissues : quinine hydrochloride in a ·09 per cent solution
gave a threefold increase in the output of carbon dioxide from
living stem apices of the broad bean but was without effect on
killed apices ; a dose of 1 per cent gave the same increased
yield from the live stems and reduced the evolution of carbon
dioxide from the dead.   Arbutin in a 1 to 2 per cent solution
depressed the respiration of wheat seedlings to a greater degree
in dead than in live seedlings.   Palladin looks upon the in-
creased output of carbon dioxide in living tissues following
poisoning as being due to the reaction of the protoplasm
against the poison employed.   In such living tissues the poison
does not result in a change in the amount of peroxide ; in
killed tissues, on the other hand, the decreased output of carbon
dioxide is accompanied by a reduction in the amount of per-
oxidase, which presumably is destroyed by the poison.*

With regard to that aspect of respiration associated with
enzymes more directly connected with the protoplasm, Pal-
ladin † adopts the view not uncommonly held ‡ that the pro-
cess is anaerobic and is essentially the same phenomenon as
alcoholic fermentation.   It has been mentioned that a supply
of respirable material, of which sugar is a prominent material, is
an important conditioning factor governing the respiration rate.

Of the enzymes able to effect the decomposition of sugar,
zymase is the most prominent ; to what extent other enzymes,
more especially those associated with respiration in the narrower
sense, are operative is a subject for further investigation.
Zymase has been described as being of widespread occurrence.
The successive stages in the reduction are not known, where-
fore agreement regarding the nature of the complete reaction
has not been reached.   Palladin considers that there is in the
first instance a reaction between the sugar and water which
leads to the formation of carbon dioxide, which is thus of
anaerobic origin, and hydrogen.   But hydrogen as such is not
one of the gaseous waste products, wherefore it must be fixed
as quickly as it is formed.   Here, Palladin's respiration pig-
ments are operative : they exist in the plant in the form of

* Palladin : " Jahrb. Wiss. Bot.," 1910, 47, 431.

† Palladin " Zeit. Gärungs. Physiol.," 1912, 1, 91. :

‡ Bach and Batelli : " Compt. rend.," 1903, 136, 1357 ; Godlewski and
Polzeniusz : " Bull. Int. Acad. Sci. Cracovie," 1897, 267 ; 1901, 277.

prochromogens, which may be glucosidal in nature; these prochromogens are converted by enzyme action into chromogens. The chromogens are oxidized by peroxidase into respiratory pigment, which oxidation, according to Combes,[*] is accompanied by an increase in the respiration intensity. These respiration pigments are the hydrogen acceptors and act in the same way as does the methylene blue in the experiments previously mentioned (p. 64): they combine with the hydrogen to reform the chromogen; the chromogen in turn and by the aid of oxidase combines with atmospheric oxygen to form water, which is thus of aerobic origin, and respiration pigment.

It will be observed that the views of Palladin and Wieland are in accordance in their regarding hydration an initial change in the oxidation of an aldehyde or a sugar. But to what extent Palladin's ideas regarding the cycle of changes involved in the complete respiratory process are accepted is a matter for individual judgment. His thesis is bound up with Bach and Chodat's hypothesis, the tenability of which is discounted by the observations of other investigators. The respiratory chromogens may act in the way outlined; but to what extent their operation is universal is very doubtful. The existence of phases, expressed in the different origins of carbon dioxide, appears to be well supported by the facts, as also is the initial hydration phase in certain oxidations. Finally, the occurrence of a fermentation stage in the respiration cycle is a strong probability if not a completely proven fact.

With regard to the consumption of fats and proteins in respiratory processes, there is but little precise information. Oparin [†] has examined chlorogenic acid, $C_{32}H_{38}O_{19}$, which he has found to occur in over a hundred different plants, and finds it to be a substance which readily oxidizes in the air, losing 4 atoms of hydrogen and forming a green pigment. The latter can act as a hydrogen acceptor and so act as an oxidizing agent. The calcium salt of the fully oxidized acid is represented by the formula $CaC_{32}H_{32}O_{19} + 2H_2O$, whilst the salt of the unoxidized acid has the formula $CaC_{22}H_{36}O_{19} + 2H_2O$. Chlorogenic acid is more particularly active in the

* Combes: "Rev. gén. Bot.," 1910, 22, 177.

† Oparin: "Biochem. Zeitsch.," 1921, 124, 90; Gorter: "Annalen d. Chemie.," 1908, 359, 217.

oxidation of natural amino acids, peptides and peptones giving origin to ammonia, carbon dioxide, and an alde-hyde.

$$R . CH . NH_2 . COOH + O \rightarrow R . CHO + NH_3 + CO_2$$
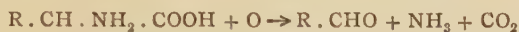
The process is accelerated by the presence of certain reagents such as phosphoric acid. Chlorogenic acid would therefore appear to be a respiratory pigment in the sense used by Palladin.

The chemical changes involved in the destruction of re-spirable material, must be to a large extent conjectural until precise knowledge is available of the relatively simple enzymic and the more complicated protoplasmic agencies employed, and of their mode of attack. For this reason it is desirable on the present occasion merely to give a brief statement of the more obvious possibilities.

If the essential part of respiration, that concomitant with life in distinction to that post mortem, is essentially anærobic —and there is much to be said for the thesis—then a sequence of chemical changes similar to, if not identical with, alcoholic respiration is indicated. Unfortunately agreement regarding the stages in this sequence has not been reached,* although the views of Neuberg and Reinfurth appear to be well sup-ported by the facts of laboratory experiment. According to these views, glucose is converted into glyceric acid and di-hydroxyacetone, each of which yields methyl glyoxal which in turn gives origin to pyruvic acid. The pyruvic acid by the action of carboxylase gives rise to carbon dioxide and to acetic aldehyde, from which ethyl alcohol results. Alcohol is an end product of anærobic respiration and its fate depends on whether or not an ærobic phase follows. In the presence of oxygen, the alcohol is oxidized to carbon dioxide and water, or it may be used up in the synthesis of protein, since by oxidation it can give origin to acetic acid which is employable in the formation of amino acids. To what extent these intermediate products obtain in the plant is not known and again it must be men-tioned that the plant is not a test tube. Indications of the sequence may possibly be obtained from animal sources and in their respiration glucuronic acid occurs and also lactic acid

* Vol. I., p. 382.

which accumulates in fatigued muscle. Lactic acid, either as such or combined in the form of a salt, has been considered an intermediate product in alcoholic fermentation, and Stoklasa and Chokensky * state that they have isolated a zymase which converts sugar into lactic acid accompanied by a lactacidase which resolves the acid into alcohol and carbon dioxide. The evidence, however, for lactic acid being an intermediate product is inconclusive and in animal physiology the facts would appear to warrant the statement that lactic acid has its origin, at any rate in part, from protein.

With regard to glucuronic acid, Spoehr has succeeded in isolating it from certain Cactaceae, the only plants so far in which it has been recorded.

The depth of our ignorance of this important aspect of respiration is obvious : and until all the possible successive stages in the dissociation of fats, carbohydrates and proteins, all of which are respirable, are known, it is impossible to trace the sequence obtaining in the living organism.

* Stoklasa and Chokensky : " Ber. deut. bot. Gesells.," 1907, **25,** 122.

CHAPTER VI.

GROWTH.

THE term growth not infrequently is used to imply mere increase of the plant or plant member in various directions with little or no attempt to correlate or to analyse these and other related expressions of the activity of the organism. Thus increase in surface is not necessarily growth : a pound pat of butter may be spread over a number of slices of bread whereby its area is increased but not its mass. An etiolated seedling may show a much greater length of internode than its fellow of the same age grown under normal conditions : but the comparison of the dry weights of the two will show no increase in mass in the etiolated example.

From considerations such as these, the conclusion is reached that growth is properly speaking an expression of the metabolism of the organism ; it is, to use F. F. Blackman's phrase, the finished product of the metabolic loom. It is this aspect which will mainly be considered on the present occasion.

Metabolism has two sides, debit and credit : if the anabolic processes are more intense than the catabolic, growth will result ; if the catabolic processes are in the ascendent, decretion obtains. From this it follows that the sure index of growth is increase in dry weight, the credit balance of the two opposite activities. Thus Boysen-Jensen [*] found that under maximum illumination the carbon assimilation of *Sinapis*, a sun plant, was 6 mgs. of carbon dioxide per 50 sq. cm. of leaf surface per hour at 20° C., whilst the respiration at the same temperature and for the same units of surface and time was ·8 mg. of carbon dioxide. This means that for an average plant of *Sinapis*, the amount of dry matter made in a day in July is

[*] Boysen-Jensen : " Bot. Tidsskr.," 1918, **36**, 219.

60 mgs. whilst the loss of dry matter due to respiratory pro-
cesses is 14 mgs., leaving a balance of 46 mgs., which is equi-
valent to 16·5 per cent. of the dry weight of the plant. A
similar relation is shown by *Oxalis*, a shade plant, but the
amounts are much smaller; ·8 mg of carbon dioxide being
assimilated per 50 sq. cm. per hour at 20° C., whilst the loss
due to respiration is ·15 mg. at the same temperature for the
same units.

A disposal to go further may be evinced and to select
the number or weight of offspring as being the true
measure of a naturally growing organism's growth, since the
selfish needs of the parent are thereby eliminated. For
obvious reasons such a measure is impracticable except in
special cases where reproduction takes place with extreme
rapidity, as for instance in bacteria, or where crop yield in re-
sponse to methods of cultivation is required.

The employment of the dry weight method has a disad-
vantage in that it forbids the study of progressive change in
one and the same member, since to find the dry weight,
the plant or plant member must be killed. For this reason
other indices sometimes must be employed. Thus change in
the size and area of leaves in certain investigations serve as a
reliable measure, a fact which becomes evident when it is
realized that an increase in the size of a leaf, or of the entire
chlorenchyma system of the plant, means an increase in the
plant's factory and all that this connotes. Thus Johnston *
found that the total dryweight and total leaf-area of the buck-
wheat ran on parallel lines during the season February to
October, the greatest rapidity of growth occurring in the
summer months.

In further illustration the observations of Briggs, Kidd, and
West,† based on the investigations of Kreusler and others on
the maize, may be considered. Briggs, Kidd, and West analyse
the growth of the maize in terms of dry weight, leaf area and
time, and employ the relative growth rate and the leaf area
ratio. The relative growth rate curve is the weekly percentage
increase in dry weight plotted against time, and the leaf area

* Johnston: " Johns Hopkins Univ. Circ.," 1917, 211.
† Briggs, Kidd and West: " Ann. Appl. Biol.," 1920, **7**, 103, 202.

ratio curve is the leaf area in square centimetres per gram of dry weight plotted against time.

The growth rate of the maize varies much in magnitude at different periods of its life. This is expressed in a generalized form in Fig. 7.

The early seedling stage is shown by the portion *ab*, and is characterized by a decretion owing to the young leaves being in a low phase of carbon assimilation activity, and providing less material than is consumed in respiratory processes, an observation which confirms the results of Irving and of Briggs. The phase *bc* corresponds to the morphological development of the
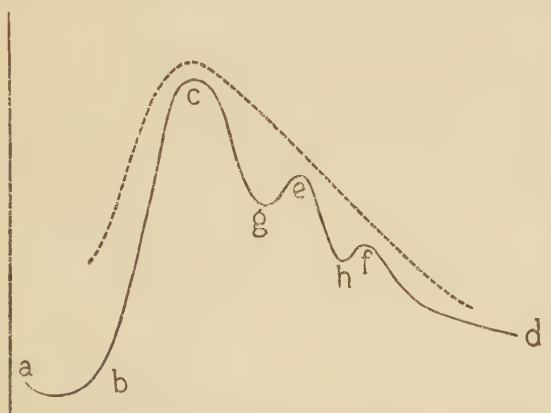
FIG. 7.—The continuous line represents growth rate and the broken line leaf area per unit dry weight.

plant during which the leaf area per unit dry weight increases to a maximum. Finally the phase *cd* is the latter part of the plant's life and includes the formation of the flower and the seed. This portion shows two secondary maxima at *e* and *f* which are respectively coincident with the appearance of the male and female inflorescences and each is preceded by a minimum, *g* and *h*, which corresponds to the early stages of flower development at which epoch there is a marked increase in respiration activity.

The incidence of the maxima is dependent on environmental conditions operating not at the time but at a previous stage in the life of the plant.

The fact that the curve for leaf area per unit dry weight corresponds with the growth rate curve indicates the close physiological connexion between the relative growth rate and carbon assimilating area per unit of dry weight (Fig. 7). The correspondence, however, is not precise ; instead of showing a definite type of variation, as does the relative growth rate, the leaf area per unit dry weight curve fluctuates about a mean value. These fluctuations are due to the conditioning factors of growth of which factors temperature is amongst the most significant. The importance of leaf area in the economy of the higher plant is so obvious that no elaboration of the statement is required. The growth in area of the leaves of the cucumber has been closely studied by Gregory * and may be alluded to at this stage in that it introduces some aspects of growth which properly belong to a general consideration. But before this is done, the " grand period of growth " must be recalled to memory. As is well known, Sachs used this expression to designate the period through which the plant, or plant member, exhibits its sequence of growth rates. Thus the growth rate of the first internode of *Phaseolus multiflorus* is at first slow, then quickens to a maximum, after which a decrease in the rate to zero obtains, which point is coincident with the attainment of maturity. The sequence is illustrated in Fig. 8 which is based on Sachs's measurements.

The grand period of growth does not include an analysis of the fluctuations in the rate during the periods between the measurements ; thus the growth rate between the third and fourth day is the summation of the growth during that period. Priestley and Evershed † in their study on the root growth of *Tradescantia* and tomato, based on the increase in dry and wet weights of the roots produced on cuttings, find the curves obtained are a sequence of these grand period curves which are a series of S curves, a type which is very common in graphs illustrating growth phenomena (see Fig. 9). The time of transition from one S curve to the next coincides with the time of appearance of a crop of roots of a subordinate branch order.

Gregory found that the growth in length, in breadth, and in area of the leaves of *Cucumis sativus* show a grand period

* Gregory : " Ann. Bot.," 1921, 35, 93.
† Priestley and Evershed : *Id.*, 1922, 36, 225.

of growth when grown under natural conditions; but when
continuously illuminated by electric light, a method some-
times adopted by market gardeners to secure early crops, the
rate of increase falls from the first measurement of area.   The
curves of increase in linear dimensions and in area can be
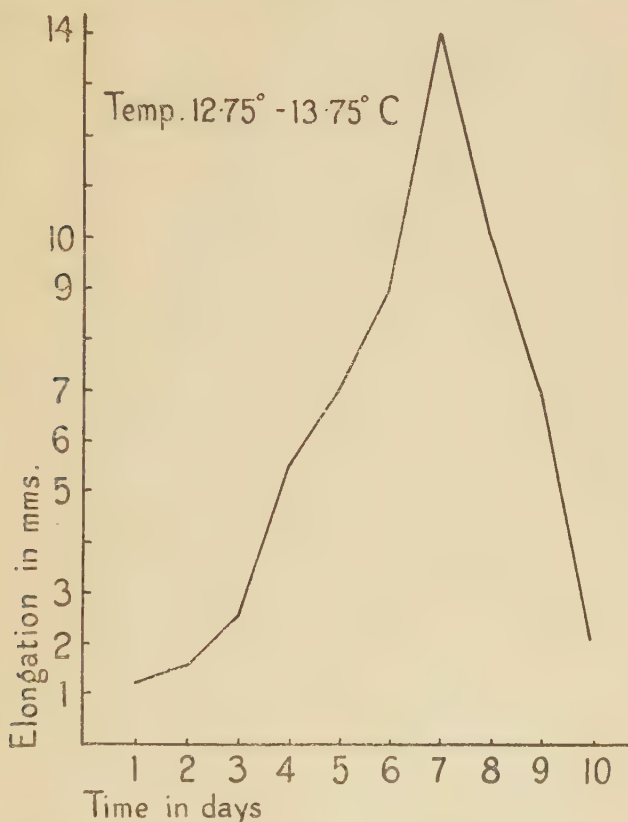
Fig. 8.

represented by the formula of an autocatalytic reaction.*   The
increase in area of the total leaf surface varies with the season of
the year: in March and June in daylight, the rate of increase
is proportional to the area existing at that time, that is, the
growth follows a compound interest law; but during December
a detrimental factor intervenes so that the rate of increase,

* This term is explained on p. 113.

although still proportional to the extant leaf area, is not maintained but falls away in time. When grown under artificial light, results comparable to those grown in daylight during December obtain. The detrimental factor is hard to formulate: in December the low light intensity and the low temperature militate against photosynthetic activity and thus the amount of available food may be adversely affected.

When grown under artificial light, the energy value of the illuminant was about equal to winter sunshine, but the temperature of the greenhouse was that of the summer, conditions which indicate that respiration was intensified whilst carbon assimilation was depressed.

In this consideration mention has been made of the law of compound interest and of the law of autocatalytic reactions.

V. H. Blackman [*] has drawn attention to the fact that in many natural phenomena the rate of change of some quantity is proportional to the quantity itself. This is the law of compound interest; money left to accumulate at compound interest increases to an amount the magnitude

FIG. 9.—Growth of the fruit of *Cucurbita pepo*; . . . observed value, x x x calculated value.

[*] Blackman: "Ann. Bot.," 1919, **33**, 353; "New Phytol.," 1920, **19**, 97. See also Kidd, West and Briggs: *Id.*, 88.

of which depends on the initial capital, the rate of interest and the time the money is accumulating ; the dry weight of an annual plant depends upon the dry weight of the food reserves in the seed, the percentage increase in the dry weight over the selected period, and the time during which the plant is increasing in weight.   This may be represented by the equation

$$W_1 = W_0 e^{rt}$$

Where W_1 is the final weight, W_0 the initial weight, r the average rate of interest, t time, and e the base of natural logarithms.

The rate of interest is obviously of first rate importance and if constant the final weight will vary directly as the initial weight, wherefore a large seed, with more initial capital, will give a much larger plant than a small seed, with a relatively smaller initial capital.

From considerations such as these Blackman arrives at the conception that the rate of increase observed in the plant is the index of efficiency of the plant, a conception which gives a useful basis for comparison but which is not a constant since it is the average of a number of rates which may show variations through a wide range, for it is affected by the external conditions.   The rate of increase is highest in the early stages of growth and falls with the inception of the reproductive period.

The laws governing autocatalytic reactions are the logical outcome of the laws of monomolecular reactions.*   An autocatalytic reaction is one in which one of the products of dissociation of the original material acts as a catalyst on the material which is obviously decreased in amount as the reaction proceeds.   Thus a solution of methyl acetate undergoes autodissociation into methyl alcohol and acetic acid ; of these products the acetic acid catalyses the methyl acetate so that the rapidity of the reaction is continually accelerated, owing to diminishing amount of methyl acetate, and the increasing amount of acetic acid, until the whole of the substrate is dissociated.

From his study of the growth of various organisms,

* Vol. I., p. 363.

Robertson * concludes that in any particular growth cycle, either of an organism or of a member of an organism, the maximum increase in volume or in weight in a unit of time occurs when the total growth due to the cycle is half completed. Such a cycle of growth conforms to the formula

$$\log \frac{x}{A - x} = K(t - t_1)$$

where x is the amount of growth in weight or volume which has been attained in time t. A is the total amount of growth attained during the cycle, K is a constant and t is the time at which growth is half completed. These relations are such as would be expected to hold good were growth the expression of an autocatalytic chemical reaction, and the growth of the organism should, from the hypothesis, remain constant, having once attained its maximum. But the contrary obtains, in old age and senescence a loss of weight occurs; this is supposed to be due to the action of secondary factors which are imposed on the phenomena of growth itself.

An inspection of Robertson's figures, for example those given for the oat,† or for *Cucurbita*, shows differences between the observed and calculated growth values of varying magnitude, and these differences when expressed in percentages sometimes appear too large to support the thesis ; but when the observed and the calculated values are expressed in the form of a curve (Fig. 9) their incidence is sufficiently close to give support to the thesis, especially when allowance is made for the experimental errors.

To what extent and in what degree growth processes may be correlated with this law is uncertain. The observations of Gregory show that the curves of increase in the area of the leaf surface of cucumber plants are of the S form and can be represented by the formula of such a reaction. In the earlier stages of growth this increase also is in accordance with a law of compound interest. There is thus a choice between the two expressions : the compound interest law is a conception rather than a physiological constant, but even so Gregory considers it to have the advantage over the law of autocatalytic

* Robertson : " Arch. Entwicklungsmech. Org.," 1908, 25, 581.
† *Id.*, 26, 108.

reactions in that it is independent of hypothetical mechanisms of growth.   For it is obvious that if growth is an expression of the activity of some catalytic agent, that agent must be sought out; according to Robertson * the lipoids may sub-serve the required function.   With regard to other observations in this aspect of the subject, the work of Reed and Holland,† who found that the rate of growth of the sunflower closely approximates the course of an autocatalytic reaction, may be mentioned.   Reed ‡ also observed that the rate of increase in the height of walnut and pear trees showed growth cycles throughout the growing season.   In each cycle the growth rate corresponded to an autocatalytic reaction.

Returning to the terms in which increase in growth may be expressed, allusion has been made to offspring especially of unicellular organisms in which the unit grows to a certain size and then divides.   The yeast plant may be selected in illus-tration, more especially as a consideration of its reproduction rate will introduce other aspects of the subject of growth.

A young yeast cell on being placed in a suitable medium increases to a certain size, the magnitude of which depends to no small extent upon the osmotic strength of the medium,§ and then reproduces itself by gemmation.   The phenomenon may be illustrated by the accompanying Fig. 10 which is based upon observations made by Slator.‖   This figure represents the offspring of a single cell up to and including the fourth generation.   The cycle was completed in 232 minutes from the second generation, the average time for the interval between one generation and the next, that is the generation time, being seventy-six minutes.

Elaborating this general statement, the growth of the yeast exhibits a sequence of phases the conspicuousness and dura-tion of which varies with the conditions.   The "seed" on being sown in the wort may remain inactive for a time; this is the lag phase, the duration of which depends in the main on the age of the seed, old plants showing a longer quiescent period than young plants grown from spores which may show

* Robertson : " Arch. Entwicklungsmech. Org.," 1913, **37**, 497.
† Reed and Holland : " Proc. Nat. Acad. Sci.," 1919, **5**, 135.
‡ Reed : " Journ. Gen. Physiol.," 1920, **2**, 545.
§ See Drabble, E. and H., and Scott : " Biochem. Journ.," 1907, **2**, 221.
‖ Slator : *Id.*, 1918, **12**, 248.

no lag phase. When once growth has started, it is unrestricted and follows the logarithmic law, for which reason this phase often is termed the logarithmic phase. This is followed by a
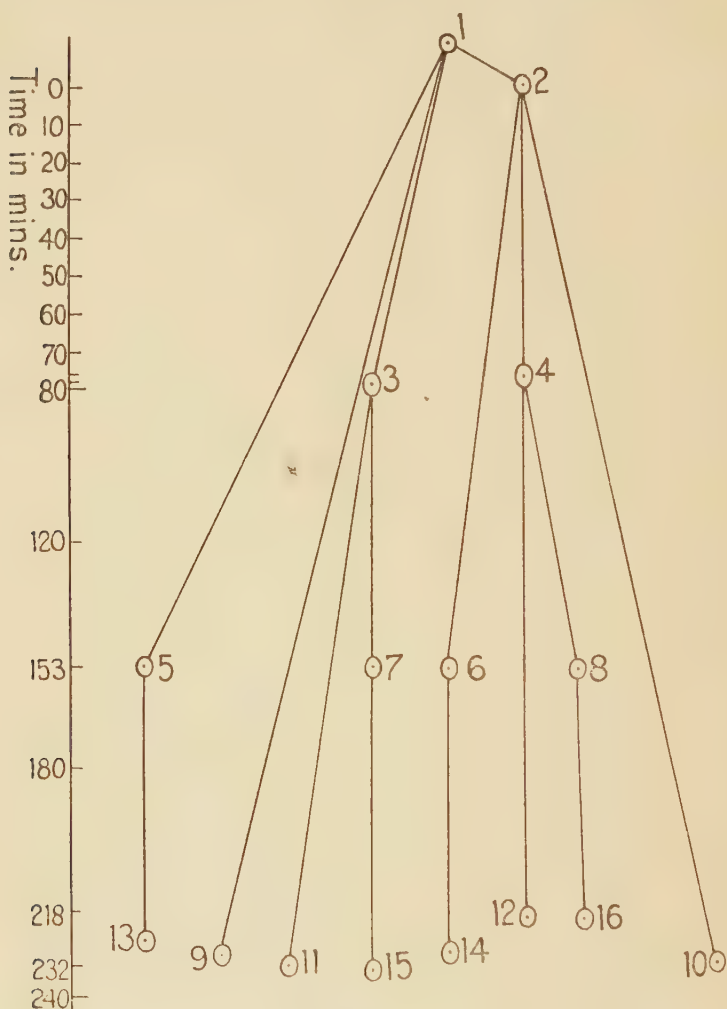
FIG. 10.

period of retardation due to the operation of factors such as the accumulation of carbon dioxide and the lack of oxygen. This is a normal sequence : but departures from the normal may result on varying the conditions of growth ; thus if the

seeding be heavy, but not excessive, the logarithmic phase may disappear and retardation set in immediately after the lag phase. If the seeding be excessive, the retarding factors may prevent any measurable growth.* Priestley and Pearsall † look upon the logarithmic phase as the natural rate of increase where increase in mass is an exponential function of time ; the retardation phase, in which the growth rate is directly proportional to time, is due to adverse operating factors, such as the accumulation of end products, which are the outcome of the earlier unrestricted growth. In addition to the obvious products, carbon dioxide and alcohol, of the activity of yeast, oxygen and sugar may also influence its growth.

The influence of oxygen is important, and this notwithstanding the fact that yeast is capable of action under anærobic as well as ærobic conditions. The growth exhibited under these different circumstances is not the same : when grown anærobically, yeast cells quickly acquire a static condition of equilibrium with regard to the medium in which they are suspended ; ‡ the lack of oxygen, especially prior to the beginning of gemmation, arresting the reproduction function. It is the small amount of oxygen initially present in the wort which is considered to explain the fact that under fixed conditions the maximum cell increase is independent of the number of cells of seed yeast per unit volume of yeast. Thus Horace Brown § found that up to 65 or 70 per cent of complete aeration, the cell increase is a linear function of the available free oxygen at the commencement of growth. In other words, during the period of active reproduction in a suitable medium in which access to oxygen is limited to that initially present in solution in the liquid and under conditions of culture which eliminate the competition factor, the number of yeast cells present at any moment is directly proportional to the time. Reproduction can, however, take place under anærobic conditions to a small extent : Horace Brown finds the limit to be 6·5 cells for each initial cell of seed yeast. This is explained by the fact that before reproduction takes place, the cells of the seed yeast absorb and fix oxygen which renders possible

* Slator : " Biochem. Journ.," 1918, **12**, 248.
† Priestley and Pearsall : " Ann. Bot.," 1922, **36**, 239.
‡ Adrian Brown : " Journ. Chem. Soc.," Lond., 1905, **87**, 1395.
§ Horace Brown : " Ann. Bot.," 1914, **28**, 197.

this limited reproduction under anærobic conditions. This absorption of oxygen, which is a linear function of time, takes place with great rapidity; thus in one instance it was found that ·3 gram of pressed yeast per 100 c.c. of liquid completely absorbed the oxygen in two and a half hours. Horace Brown concludes that the power of reproduction is impressed in the cell at the very outset by the absorbed oxygen and that a quantitative relation exists between this absorbed oxygen and the number of units which the initial yeast cell can finally gemmate. The action of the oxygen is one of induction and, according to Horace Brown, all the known facts can be explained on the assumption that the available oxygen is equally divided between the initial cells and the consequent variation in the oxygen charge which these cells must receive when the ratio of the seed yeast to the available oxygen varies.

The amount of oxygen in aerated wort may be very small but its effect may be very great: thus 1 c.c. of oxygen in aerated wort brings about a growth sixty times greater than the same amount of oxygen in nonaerated wort.* Slator is impressed by the importance of carbon dioxide as a conditioning factor in the growth of yeast and he considers that the influence of this gas is much greater than is generally supposed, in fact that some of the observed effects generally ascribed to the direct influence of oxygen may be due to its indirect action in lessening the supersaturation of the wort with carbon dioxide. For measurements of the rate of growth in wort and in wort saturated with carbon dioxide show much retardation, possibly due to the carbon dioxide rather than to the lack of oxygen. These measurements are confirmed by controlled experiments in which the carbon dioxide was the limiting factor. In fact, a correlation can be made out between the crop of cells and the concentration of carbon dioxide in the medium :—

Proportionate Concentration of Carbon Dioxide = a.	Crop of Cells. †	$\dfrac{Crop}{a}$.
1·08	3·0	2·8
1·08	3·0	2·8
1·46	4·1	2·8
1·48	4·5	3·1
2·13	6·1	2·9
2·33	6·5	2·8

* Slator: "Journ. Chem. Soc.," Lond., 1921, **119**, 115.
† Unit of crop of cells = $7·65 \times 10^6$ cells per c.c.

With regard to the influence of sugar, there is, according to Slator, a proportion between the size of the crop and the initial concentration of glucose up to about 1 per cent. The accompanying curve (Fig. 11) shows the retarding influence of sugar in increasing concentration, when sugar is the limiting factor. Its slope corresponds to 3900×10^{16} cells per gm., a figure in fair agreement with the calculated number.

With respect to alcohol, under ordinary conditions of brewing its accumulation is never so great as to be a significant factor in the growth of the yeast; if, however, alcohol be present
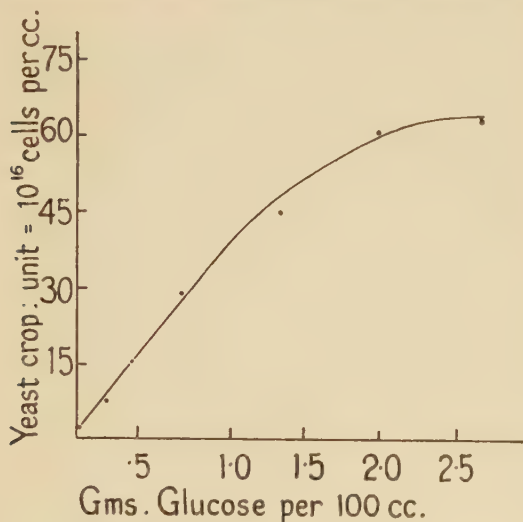
FIG. 11.

in excessive amount, it has a limiting action; thus the presence of 8 per cent of alcohol in the fermenting liquor may inhibit reproduction, especially if the supply of oxygen is limited.

This survey, incomplete though it be, shows the usefulness of offspring as an index of growth * and emphasizes the importance of various conditioning factors in the governance of life processes.

The growth rate of a plant varies with the age of the organism and also may show periodic and seasonal variation.

* A little thought will show that measurements of increase in length and girth of those parts of plants exhibiting merismatic activity are in reality measurements of offspring of the dividing elements.

Eucalyptus regnans shows the greatest rate of increase of area between the age of forty and fifty years.* The measurement of the diameter of the annual rings indicates that the growth rate falls off with time. In many instances growth in thickness is periodic through the seasons on account of climatic factors, thus at Peradeniya *Hevea brasiliensis* shows no growth in thickness during the dry season January to March. From the end of March to the beginning of October, the wet season, growth is uniform; whilst during the dry season October to December growth is considerably less and sometimes may cease altogether.† In instances such as this it would appear that climate is all-important, especially as regards the provision of adequate supplies of soil water. Thus the erratic growth of *Hevea* observed during the second dry season may be predetermined by the amount of rainfall during the previous wet season.

In more temperate climates, soil temperature is a significant factor; the observations of McDougall‡ show that the root growth of forest trees begins in the early part of the year when the soil temperature reaches a degree sufficiently high for absorption of water to take place, and stops in the autumn when the soil becomes too cold. There is no inherent tendency for periodicity, and when a resting period obtains during the summer months, its cause may be found in the decreasing water supply. There is, however, as Petch has shown for *Hevea*, a personal as well as a specific physiology of plants, an almost untrodden field, and many of the results observed may be ascribed to personal peculiarity rather than to this or to that factor.

Allusion has been made to the importance of the previous history of the conditions and of the plant in determining the activity of the plant at the present moment. In the present connexion, the important and extensive work of Balls on the cotton plant must be mentioned.§ From a long series of observations, Balls concludes that the different behaviour of the plants, as indicated by the crop of cotton, are the inevitable

* Patton: " Proc. Roy. Soc. Victoria," 1917, **30,** 1.
† Petch: "Ann. Roy. Bot. Gard. Peradeniya," 1916, **6,** 77.
‡ McDougall: " Amer. Journ. Bot.," 1916, **3,** 384.
§ Balls: " Phil. Trans. Roy. Soc.," Lond., B., 1917, **208,** 157.

consequences of the known environmental conditions, provided that proper regard is paid to the distinction in time between the incidence of the conditioning factor and its manifestation in the crop.   Thus the daily fluctuations in the flowering curve are predetermined and controlled by the weather conditions which obtained a month before the flower opens.   This is the principle of predetermination : its importance is obvious ; an accurate knowledge of predetermining factors will amongst other things give to certain aspects of physiology, ecology and agriculture a precision which now is sometimes lacking.

THE CONDITIONING FACTORS.

TEMPERATURE.—The factors which condition growth and its rate are precisely those which influence anabolic and catabolic activities, and the effect of any one such factor on growth is the resultant of its action on the opposing components of growth.   Thus temperature accelerates respiration and also carbon assimilation, but since in a vigorous green plant the products of one hour's carbon assimilation is a sufficient provision for many hours' respiration,[*] there will be a balance on the credit side ; wherefore an increased temperature will result in an increased rate of growth, other things being equal.   But there is a limit to the height of the temperature ; if a certain degree be exceeded, growth will be retarded, come to a halt, and ultimately a decrement will obtain.   In illustration, it is a common laboratory experience that the increase in length of the radicle of a seedling is a linear function of time and that there is a gradual increase in the rate of growth up to about 28° C.   Talma [†] observed the growth of the radicle of *Lepidium sativum* from 0° to 40° C.   Under conditions of constancy of temperature for at least three and a half hours, it was found that measurable growth obtained at 0° C. and that the greatest rate occurred at 28° C.   Van't Hoff's law is applicable only for small ranges of temperature and, in general terms, the temperature coefficient decreases with a rising temperature.

[*] See Boysen-Jensen : " Bot. Tidsskr.," 1918, **36**, 219.
[†] Talma : " Koninkl. Akad. Wetensch.," 1916, **24**, 1840.

The same general observations have been recorded for lower plants. Thus Fawcett * found that the radial growth rate of cultures of the fungus *Pythiacystes atrophthora* remained constant as long as the environmental conditions did not change. With increasing temperature a greater rate of growth is exhibited up to a certain degree; when this is passed, the rate of growth falls off. Thus :—

At 10° C., radial growth rate = 2·5 mm. per 24 hours.
" 20° C.,   ,,   ,,   ,, = 6·0 ,,   ,, ,,   ,,
,, 28° C.,   ,,   ,,   ,, = 7·5 ,,   ,, ,,   ,,
,, 33° C.,   ,,   ,,   ,, = 2·6 ,,   ,, ,,   ,,

Balls † measured the growth in the length of the hyphæ of the fungus causing "sore skin" on the cotton plant. The growth rate at different temperatures is what might be expected from van't Hoff's law; but at higher temperatures, 38° C., there is a decrease in growth followed by complete cessation probably due to the accumulation within the cell of certain products of catabolism; these deleterious substances presumably are formed at lower temperatures but at a much slower rate; they diffuse out into the surrounding medium, especially at higher temperatures, possibly on account of alterations in the permeability of the protoplasm at these higher temperatures.‡ In the case of higher plants, the outward diffusion of these harmful bodies, provided they be formed, must be slower on account of the more massive nature of the structures, or they are oxidized within the tissues themselves.

As Balls points out, since the conditions under which this decomposition takes place must be fairly uniform in a higher plant, growth optima are shown which are the expressions of the internal struggle between the increasing rapidity of chemical change with the rise in temperature and the inhibitory action of the accumulating products of catabolism.

The investigations of Leitch § on the influence of temperature on the rate of growth in the roots of *Pisum sativum*, show that the relationship can be expressed as a uniform

* Fawcett : " Johns Hopkins Univ. Circ.," 1917, 193.
† Balls : " Ann. Bot.," 1908, 22, 557.
‡ See Eckerson : " Bot. Gaz.," 1914, 58, 254.
§ Leitch : " Ann. Bot.," 1916, 30, 25.

curve for the range of temperature $-2°$ C. to $29°$ C. and
resembles those of Kuijper for respiration.   Above $29°$ C. there
is so much fluctuation that relationship cannot be expressed
in a single curve, wherefore for each higher temperature a
different curve must be made to express the rate of growth
in successive periods of time.   This is owing to the operation
of one or more of those imperfectly known factors termed by
F. F. Blackman the time factor.   For instance, at $30°$ and $35°$ C.
the rate of growth in the first ten minutes is the highest at-
tained, in the first half hour there is a rapid fall followed by a
recovery marked by a rise to a second maximum, after which
there is a gradual fall.   At $40°$ C., the decrease in growth
rate is uniform and rapid, there being no recovery.*   As in
the cress, observed by Talma, so in the pea ; the coefficient
for a rise in the temperature of ten degrees shows a distinct
falling off as the temperature rises, and, according to the
observations of Leitch, it is only between $10°$ and $22°$ that
the coefficient value lies between 2 and 3, for which reason
the complete curve is not regarded as a van't Hoff curve.
The extremes of measurable growth was observed at $-2°$
and $44·5°$ C., the highest rate being at $30·3°$ C.   With re-
ference to these observations, Leitch distinguishes four cardinal
points : the minimum temperature, the maximum temperature,
the optimum temperature and the maximum rate temperature.
The minimum temperature for any physiological process is
the lowest temperature at which the process takes place ;
the maximum temperature is the highest temperature at which
the process takes place ; the optimum temperature is the highest
temperature at which there is no time factor operating ; and
the maximum rate temperature is that temperature at which
the process attains its highest intensity.

The fluctuations observed at higher temperatures may be
due to several factors ; under such conditions some bio-
chemical reactions may be intensified but not necessarily at
the same rate ; and some may be inhibited in varying degrees.
The operations of enzymes, for example, are not affected in
precisely the same way by the same high degree of tempera-
ture.   Such a contingency may interfere with the supply of

* See also Sierp : '' Ber. deut. bot. Gesells.,'' 1918, **35**, 3 ; '' Bot. Zentrbl.,''
1920, **40**, 433.

soluble food, and, concurrently, the rapid accumulation of the
products of respiratory processes, which also are accelerated by
an increased temperature, may have a toxic action. In a few
words, up to a certain degree a rise in temperature accelerates
physiological actions and here a Van't Hoff curve may be
expected : beyond this degree, co-ordination becomes less and
less, wherefore metabolic derangement obtains. Priestley and
Pearsall,* in their examination of Leitch's results, point out
that the growth rate of radicles is dependent on the chemical
reactions involved in the merismatic activity of the growing
point, and on the hydrolysis of the reserve food in the seed and
the translocation of the products to the growing parts.

The situation therefore is this : the increase in temperature
accelerates both growth and hydrolysis, but the merismatic
tissue quickly uses up the food immediately available, wherefore
a decrease in the rate of growth must ensue for that period of
time taken by the food materials in their translocation from the
seed to the apical regions of the root. The arrival of this food
accounts for the second maximum in Leitch's curves. Finally
the growth rate is diminished by the dislocation of the meta-
bolic processes. If this contention be correct, a close correla-
tion between the length of the root and the time of the incidence
of the second maximum should obtain.

LIGHT.—The influence of light on growth is a subject of
considerable magnitude, especially when the term growth is used
in its general sense : the directive action of light in tropistic
and kindred phenomena, its influence in the determination of
growth form and the facies of a flora are aspects of the subject
outside the scope of the present consideration.

The action of light on growth is both direct and indirect,
and its action is most marked in those members which use
light as a source of energy. Thus for the ordinary green
plant, increase without light is an impossibility since light is
the source of energy for the making of food ; in non-green
members of plants, in total parasites, and saprophytes, on the
other hand, light, for obvious reasons, is not a factor of
consequence. It is a laboratory commonplace to find that
for the subjects generally used for experimental purposes, light

* Priestley and Pearsall : " Ann. Bot.," 1922, 36, 239.

influences growth, as indicated by its external expression of increase in length and area, in various ways.   Thus internodes in darkness attain a much greater length than in light ; leaves may develop hardly at all in darkness, as in the instance of the pea, whilst in other cases, the wheat for example, the leaves attain a size more or less equal to that in light.   This difference in behaviour is apparently due to the amount of carbohydrate, relatively large in the wheat and relatively small in the pea, available for structural purposes.   In passing, attention may be recalled to the fact that the humidity of the atmosphere is an important factor in the configuration of a plant, wherefore in experiments on living plants in closed chambers, allowance must be made for the humidity conditions.

With regard to the different qualities of light, it is commonly accepted that growth is promoted by the blue and violet rays whilst those less refrangible retard.   Here again care must be taken to ensure a just comparison : to judge the effect of, say, blue light and red light, the intensities of each must be the same or, at any rate, known ; further, in such an experiment due allowance for the different heating effects of light of different wave lengths must be made, and in some cases, if not in all, the internal temperature must be observed rather than the temperature of the surrounding medium.

The problem presents three aspects : intensity, duration, and quality.   Sierp [*] found that the general effect of an increase in light intensity is to accelerate the rate of growth of the coleoptile of *Avena sativa* and to shorten the time within which the maximum rate occurs and that the incidence of this maximum is earlier as the light intensity increases.   The net result is that the total growth is reduced as the intensity of light increases.

With regard to the effect of the duration of light on plant growth, Garner and Allard [†] conclude from their observations on the tobacco, soy, and other plants that the amount of vegetative growth is proportional to the duration of exposure to daylight, short exposures resulting in small slender plants exhibiting a slower rate of growth.   They also find that the

[*] Sierp: " Ber. deut. bot. Gesells.," 1918, **35,** 8 ; " Bot. Zentrbl.," 1920, **40,** 433.

[†] Garner and Allard: " Journ. Agric. Res.," 1920, **18,** 553.

duration of light is an important factor in instituting the reproductive phase; by modifying the periods of exposure, bienniels may be made to complete their life histories in a few months, and, on the other hand, annuals may be converted into bienniels.

In this connexion the results obtained by Blaauw [*] and of Vogt [†] from their studies on the growth of the sporangiophore of *Phycomyces* and on the coleoptile of *Avena* respectively are important. According to Blaauw, the effect of light on growth is an acceleration followed by a retardation to a rate lesser than the normal followed by a gradual increase to the normal rate. The time of incidence of the initial acceleration varies with the intensity of the illumination; thus on exposure to a light of intensity of one unit, the acceleration begins in about eight minutes, but in an intensity of fifteen units, the acceleration begins in about three and a half minutes. The amount and duration of the reactions vary with the degree of illumination: for the lower light intensities the total acceleration of growth exceeds the total retardation; and for the higher light intensities the total retardation exceeds the total acceleration. For the former, Blaauw finds that the increased growth is proportional to the cube root of the amount of light. These results are extended by Vogt, who not only finds the same acceleration and retardation in the growth of the coleoptile of *Avena sativa*, but also a considerable initial decrease in growth rate on temporary exposure to a sufficiently strong illumination. In this instance, therefore, there is first a rapid decrease, immediately followed by an acceleration, which is in turn followed, especially under increased or more protracted illumination, by a second inhibition phase which exceeds the previous acceleration; hence the total effect may be a considerable fall in the rate and in the amount of growth. For a given reduction in growth, the product of the light intensity and its duration is a constant. Gregory [‡] also found that under comparable conditions the average leaf area of the cucumber is determined by the product of the intensity and the duration of the light radiation.

The initial decrease in the growth rate is considered by Vogt to be due to the action of light alone, not to the combined

[*] Blaauw: "Zeitsch. Bot.," 1914, **6**, 641.
[†] Vogt: *Id.*, 1915, **7**, 193.
[‡] Gregory: "Ann. Bot.," 1914, **35**, 97.

effect of alternating light and darkness nor to increased transpiration.

The following observations of Vogt illustrate the photo-tonic reaction : when the coleoptile of the oat was alternately illuminated by light of the same intensity, and darkened for periods of fifteen, thirty, and sixty minutes, lesser growth was found to obtain only in the two latter periods of illumination. In these instances the greater growth in darkness is considered to be due to the stimulation of the previous exposures to light. The slower growth in periods of illumination is merely a part of the complete reaction, an acceleration being followed by a depression.

From these investigations it is clear that the reaction of the plant to light is very complex ; that the total effect observed is the balance of the measures of the accelerating and depressing influences of light ; and that the magnitude of the effect depends upon the intensity and the duration of light.

These observations refer to ordinary white light; analysed white light has effects on growth according to the particular rays and to the physiological peculiarity of the species.

Thus Schanz * grew *Begonia*, *Cucumis*, *Lobelia*, *Petunia*, and other plants in frames arranged in a series of eight ; by means of glass of different opacities, all but the first, which was uncovered, were illuminated by light from which certain rays had been abstracted :—

  1. Unaltered day light.
  2. Rays longer than 320 $\mu\mu$.
  3. Rays longer than 380 $\mu\mu$.
  4. Rays longer than 420 $\mu\mu$.
  5. Rays longer than 560 $\mu\mu$.
  6. Yellow light.
  7. Green light.
  8. Blue-violet light.

The plants mentioned showed a regular increase in length from 1 to 5 inclusive and a decrease from 6 to 8 inclusive. The behaviour of all plants, however, was not the same ; the beetroot and the potato developed better in the blue-violet than in the green, and better in the green than in the yellow.

* Schanz : " Ber. deut. bot. Gesells.," 1919, **37**, 430.

In general terms, the more the short rays, especially the ultra-
violet, were removed, the greater the growth up to a certain
limit.   It also was observed that earlier flowering was pro-
moted by culture under 3 and 4 of the series.

With regard to energy other than light, field experiments
with wheat and other crops show the beneficial action of
overhead electric discharge.*   In the year 1915 the increase
in grain and in straw was 30 and 50 per cent respectively
greater than the control, and in 1916 the corresponding figures
were 49 and 88 per cent.   The effect of the discharge also
showed itself in the year subsequent to its application ; thus
in the clover and grass crop there was a marked increase in
1916, the year following the treatment.   The reasons for the
increase following the stimulation have not yet been found.

WATER.—Common experience shows that growth is only
possible provided that the living cells are in a turgid condition,
wherefore circumstances which promote this condition will
promote vegetative growth, but not necessarily reproduction.
The circumstances to which allusion is made are humidity,
adequate soil water, absorption of water and transpiration.   A
humid atmosphere promotes vegetative growth in that it re-
duces transpiration ; adequate soil water is necessary if trans-
piration be high ; the absorption of water and its conveyance
to the transpiring surfaces is in part a question of osmosis and
this is bound up with permeability.   Although biophysical
problems are outside the present province, it may be mentioned
that any factors which interrupt the normal osmotic adjust-
ments of a plant must adversely affect its vegetative growth.
Thus Dufrénoy has pointed out † that growth is a function of
at least six factors and a variation in any one of these will
influence the velocity of growth.   He considers that growth
rate depends upon the balance between the sum of imbibition,
the osmotic pressure of colloids and of the salts of the cell sap,
and the sum of the tension of membranes, the osmotic pres-
sure of the salts of the surrounding medium and the mechani-
cal resistance of the medium.

* Blackman and Jörgensen : " Journ. Board Agric.," 1916, **23**, 671 ; 1917,
24, 45.
† Dufrénoy : " Rev. gén. Sci.," 1918, **29**, 323.

The vegetative and reproductive phases of a plant are antagonistic, for which reason conditions which promote the one will degrade the other. Thus generally a low degree of humidity favours flower production ; red light rays accelerate the formation of reproductive shoots whilst blue rays retard, but darkness does not inhibit the formation of flowers at low temperatures, it is only at higher temperatures that intermittent light and darkness can be made to effect the inhibition.[*]

NUTRITION.—The importance of food is so obvious a factor governing growth that its consideration would appear to be unnecessary : but the significance of certain raw materials, more especially inorganic substances, is so marked as to warrant some mention.

The following table, results obtained at Rothamsted,[†] generally illustrates the action of various chemical fertilizers on field crops :—

WHEAT.—(Average yield 1852-1912).

	Grain in Bushels.	Straw in Cwts.
Complete minerals	32·1	32·9
Ammonium salts + superphosphate . . .	22·9	22·3
Ammonium salts + superphosphate + sodium sulphate	29·1	28·0
Ammonium salts + superphosphate + potassium sulphate	31·0	31·5
Ammonium salts + superphosphate + magnesium sulphate	28·8	28

GRASS.—(Cut for hay every year).

	1918.	1919.	1920.
Complete mineral manure	45·2	32·9	46·0 cwts.
Minerals without potash	25·9	19·7	27·3 ,,
Complete minerals + ammonium salts .	46·7	53·2	49·6 ,,
Minerals without potash + ammonium salts	33·5	34·5	32·6 ,,

In this connexion the relative abundance of colloids in the soil would appear to be of importance ; thus Jennings[‡] finds that colloids added to the culture medium promoted or depressed the growth of wheat seedlings according to the specific colloid employed and the concentration of the culture medium. Agar, for instance, in the presence of low concentrations of

[*] See Klebs : " Flora," 1918, 11, 128.
[†] For these we are indebted to Dr. W. Brenchley.
[‡] Jennings : " Soil Science," 1919, 7, 201.

nutrient salts increases growth, but in high concentrations a depression obtains. With regard to inorganic colloids, ferric and aluminium hydroxides depress whilst colloidal silica increases growth, doubtless because silica is much absorbed by the cereal grasses and possibly in this form. In view of the adsorption properties of colloids, the depressing action of some examples may be due to their adsorbing mineral salts and thus rendering them unavailable for the use of the plant.

Further, in considering the action of inorganic compounds on growth, certain important aspects are to be remembered : the plant has a specific physiology, a good crop of nettles indicates a high nitrogen content in the soil ; the inter-relationship of the various compounds concerned, carbohydrate and nitrogen for instance ; and the fact that conditions which favour vegetative growth are not necessarily those for reproductive activity. An abundance of inorganic salts in the soil favours vegetative activity whilst a relatively small salt supply is inductive to reproduction ; but this only obtains provided that other conditions are satisfactory. Thus increase in growth is impossible for the green plant if carbon assimilation be of a low order of intensity, since carbohydrate is required for many purposes, structural, respirative, and as raw material for the elaboration of other compounds such as proteins. And for this last purpose nitrogen also is necessary ; wherefore intense carbon assimilation in the absence of nitrogen-containing substances cannot lead to a growth commensurable with the intensity of carbohydrate formation. In fact, there is between nitrogen and carbohydrate a correlation, and growth is affected according to their ratio. Thus if carbon assimilation be increased by growing plants in an atmosphere enriched by the addition of carbon dioxide whilst the nitrogen-containing salts of the soil are not increased, the ratio C/N is high and the reproductive phase is induced ; if, on the other hand, the ratio C/N is low, the vegetative activity is intensified.[*] These results may, however, not obtain if the ratio mentioned runs to extremes. Kraus and Kraybill [†] in their experimental work on the tomato found that a very high C/N ratio results in but little vegetative growth and but poor reproduction ; a

[*] Fischer : " Gartenflora," 1916, **65**, 232.
[†] Kraus and Kraybill : " Oregon Agric. Exp. Sta.," 1918, Bull. 149.

medium ratio gives moderate vegetative growth and good reproduction; and when the C/N ratio is very low, a vigorous vegetative growth and poor reproduction obtains. Thus the best results occur when these two factors are reasonably balanced. From the present aspect the value of these observations lies in the fact that they provide another instance in the interaction of the various factors involved in growth: there is no virtue in increasing the one without duly considering the others; heavy manuring with nitrates, for instance, is mere waste if there be not adequate supplies of water, and adequate and proportionate supplies of both will not promote fruit formation in a greenhouse so dimly illuminated as to depress carbon assimilation.

AUXIMONES.—Bottomley [*] concluded from a large number of experiments that something more was requisite for the vigorous growth of a plant than is contained in the ordinary culture solution made up with mineral substances. These promoters of growth, the nature and composition of which are unknown, he termed auximones. Bottomley selected such plants as *Lemna*, *Salvinia*, and *Azolla* which normally lead an aquatic existence and thus avoided the rather artificial condition inseparable from the cultivation of a terrestrial plant in an aqueous medium. For healthy growth small amounts of organic matter are necessary: amongst the best results obtained were those in which an aqueous extract of bacterized peat had been added, but other organic substances, such as autoclaved *Azotobacter* and crude nucleic acid derivatives from raw peat, will also serve.

Bacterized peat is sterilized raw sphagnum peat decomposed by nitrogen fixing bacteria of the soil; such treated peat is considered to act either as a food substance or indirectly as an accessory food substance. The amount necessary for a positive result is so small that a body comparable to a vitamin is suggested. Thus Rosenheim [†] found that plants of *Primula malacoides* treated with an aqueous extract of ·18 gram of

---

[*] Bottomley : " Proc. Roy. Soc.," Lond., B., 1917, **89**, 481 ; " Ann. Bot.," 1920, **34**, 345, 353. See also article in " The Exploitation of Plants," Ed. by F. W. Oliver, London, 1917. Mockeridge : " Proc. Roy. Soc ," Lond., B., 1917, **89**, 508.

[†] Rosenheim : " Biochem. Journ.," 1917, **11**, 7.

bacterized peat grew taller than untreated plants. This aqueous extract contained 20 mgs. of organic matter of which only 1·9 mg. represented nitrogen.

Possibly auximones are connected with the synthesis of complex nitrogenous molecules, for their action on the nitrogen cycle organisms is to increase the rate of nitrogen fixation and nitrification and to depress the rate of denitrification. There is no doubt that the use of bacterized peat may give marked positive results in pot cultures, but to what extent the treatment is advantageous to field crops is doubtful.*

HORMONES.—The study of regeneration, correlation, polarity, and cognate subjects † leads to conclusions in some respects indefinite in that no tangible factor is discoverable that will account for the beginning or for the control of certain phenomena. A cambium cell divides ; the daughter cell destined to become a permanent tissue element will develop into a phloem element if cut off on one side and into an xylem element if cut off on the opposite side ; what is it that determines the fate of the cell ? The leader of a spruce is negatively geotropic, the lateral branches are diageotropic ; if the leader be removed, a lateral branch from the topmost whorl will change its habit, become negatively geotropic and carry on the functions of the leader. Why must the leader be removed before a change in tone of a plagiotropic shoot can be effected ? The primordium of a lateral bud is laid down and, apparently, all conditions are favourable for development, yet the bud remains dormant until, say, the apex of the main shoot is removed, then the bud will immediately start its development. It is true that in many cases the diversion of food will account for the subsequent phenomena but in other instances such an explanation is inadequate and in such examples the question is : What is it that presses the trigger ?

The subject properly is beyond the scope of an introduction to the physiology of metabolism of plants but the introduction

---

* See Russell : " Journ. Board Agric.," 1917, **24**, 11.
† See Bohn : " Compt. rend. Soc. biol.," 1918, **81**, 220. Farmer : " New Phytol.," 1903, **2**, 193, 217. Goebel : " Biol. Zentrbl.," 1916, **36**, 193. Lang : " Brit. Ass. Rep.," 1915, 701. Loeb : " Bot. Gaz.," 1918, **65**, 150 ; " Journ. Gen. Physiol.," 1919, **1**, 337.

is desirable even though the acquaintance be not cultivated until some future occasion.

For long it has been known that very small quantities of various materials act as powerful stimulants ; the extraordinary effect of minute traces of zinc on the growth of moulds may be instanced. Animal physiologists recognize the effect of traces of substances in stimulating various activities, especially those associated with secretion. These substances are produced in one organ and stimulate another organ to which they are conveyed by the blood. Hence the idea of a chemical messenger, or, to use the current term, hormone.

Errera * was amongst the first of botanists to suggest that hormones play a part in the economy of the plant ; his explanation of the changes in the spruce instanced above is that the apical shoot continually is secreting an inhibiting substance which is distributed to other parts of the plant, keeping them in their normal tone. The removal of the leader of the spruce removes the source of the inhibiting hormone, wherefore a near lateral shoot assumes the qualities and functions of the lost leader.

This idea has been adopted by Loeb † who concludes from many observations on the development of buds and roots on the leaves of *Bryophyllum calycinum* that the apical bud secretes a hormone which inhibits the development of buds more basal in position. It is not until the apex is removed that the buds below will develop.

The degree of inhibition depends on the amount of hormone and the mass of the lateral bud ; it is presumably for this reason that the inhibition may only extend to these primordia situated more immediately below the apex.

This account, brief though it be, will give some idea of the action of hormones ; the hypothesis, in so far as it affects plants, however, is not universally accepted. Thus Fyson and Venkataraman ‡ can find no evidence in favour of the existence of such bodies, and Child and Bellamy § consider that inhibition

* Errera · " British Ass. Rep.," 1904, 814. See also Armstrong : " Ann. Bot.," 1911, **25,** 507.

† Loeb : " Bot. Gaz.," 1915, **60,** 249 ; 1916, **62,** 293 ; " Science," 1917, **46,** 547. See also Reed and Halma : " Plant World," 1919, **22,** 239.

‡ Fyson and Venkataraman : " Journ. Indian Bot.," 1920, **1,** 337.

§ Child and Bellamy : " Science," 1919, **50,** 362.

is a question of the conduction of stimuli rather than the movement of tangible substances. Indeed, the evidence regarding the occurrence of hormones in plants is nothing like so conclusive as the proof of their existence in the animal ; actually, hormones have never been demonstrated in the plant ; their presence is inferred in order to explain certain very complicated physiological processes otherwise inexplicable.

VITAMINS.—During recent years increasing attention has been given to a class of substances known as vitamins, or accessory food factors, which play a highly important part in the dietary of man and animals since their deficiency or absence may give rise to a variety of so-called deficiency diseases such as rickets, beri-beri, and scurvy.

Three vitamins are at present recognized and are known as fat soluble or vitamin A, water soluble or vitamin B, and a third which may or may not be identical with one of the other two. As none of these substances have as yet been isolated in a state of purity, no definite knowledge exists regarding their chemical composition, and for this reason they are frequently designated by the function which they appear to perform in the economy of life. Thus vitamin A is described as the growth-promoting or anti-rachitic vitamin, whilst vitamin B is known as the anti-neuritic or anti-beri-beri vitamin, and the third, for convenience, is known as the anti-scorbutic vitamin.

The present occasion is not appropriate for a consideration of the significance and properties such as thermostability resistance to oxidation,* and the chief reason for drawing attention to vitamins here is the fact that they appear to be produced only in the vegetable world ; in every case the vitamin content of animal tissue is dependent on the animal's nutrition with plant material containing the requisite substance ; no animal is able independently to produce vitamins for its own use.

Fat soluble A or the growth-promoting vitamin is, as its name implies, closely associated with fats or oils and is found in the animal kingdom in the largest quantities in the oils of the livers of fish which, in all probability, derive them from

* See " Medical Research Committee " No. 38, H.M. Stationery Office, 1919.

the algæ and other plants which form an important part of the diet of fish.* Amongst animal fats which are poor in vitamin may be mentioned lard, whilst suet contains a fair proportion. In view of the fact that the plant is the sole source of vitamin, it is surprising to find that most vegetable oils obtained from plant seeds have a low content of vitamin A, in fact the storage organs of plants, and especially seeds, contain little of this substance.† On the other hand, it has been shown by Drummond and Coward‡ that large amounts of this substance are formed in green leaves, where it appears to be associated with the unsaponifiable portion of the fats rather than with the protein, and that leaves deficient in chlorophyll apparently are unable to effect its synthesis. Amongst the marine algæ, the green synthesize most, the red being much less active. Fungi produce no vitamin A.

The suggestion has been made that a probable relation exists between vitamin A and carotinoids such as carotin and xanthophyll,§ a view which has been disputed and, in fact, a quantitive association between vitamin A and the yellow pigments in the plant tissue is denied.‖ Further, there appears to be no definite relationship between vitamin A and lipochromes in oils and fats.¶

With regard to vitamin B, the anti-neuritic or anti-beriberi vitamin, it has been shown that this substance is associated with the pericarp of rice and other grains and the germ of wheat and rye. The proof of this is furnished by the fact that the disease of beri-beri is associated with a diet consisting almost exclusively of polished rice or white wheaten flour in the preparation of which the pericarp and germ are respectively removed, whereas rye flour and whole meal, in which the germ is not entirely removed by milling, retain the active principle. Whole potatoes, barley and beans also contain the anti-beriberi vitamin and the inclusion of these substances or of milk in the diet ensures immunity from this disease.

* Hjort : " Proc. Roy. Soc.," Lond., B., 1922, **93,** 440.

† Drummond and Zilva, "Journ. Soc. Chem. Ind.," 1922, **41,** 125T.

‡ Drummond and Coward : " Biochem. Journ.," 1921, **15,** 530.

§ Steenbock : " Science," 1919, **50,** 352 ; " Journ. Biol. Chem.," 1921, **46,** 32 ; **47,** 303 ; 1922, **51,** 63.

‖ Palmer : " Science," 1919, **50,** 1 ; Palmer and Kennedy : " Journ. Biol. Chem.," 1921, **46,** 559.

¶ Drummond and Coward : " Biochem. Journ." 1920, **14,** 668.

Vitamin C or anti-scorbutic vitamin, on the other hand, is associated more especially with growing plants for, whereas dried peas and lentils have no effect in preventing or in curing scurvy, the same materials, if allowed to germinate, are very effective anti-scorbutics, which shows that the active material or vitamin is only secreted by the growing plant and is not contained in the seed as such.

It has been stated above that there is no definite knowledge concerning the chemical constitution of the vitamins: various suggestions have, however, from time to time been put forward only to be refuted by subsequent work. At the moment, two views are in the field: the one held by Williams[*] is that they are related to hydroxypyridine and contain a betaine ring, the other, that of Besszonoff[†] is that they are polyphenolic substances related to hydroquinone.[‡]

[*] Williams: Journ. Biol. Chem.," 1917, 29, 495.
[†] Besszonoff: " Compt. rend.," 1921, 173, 466.
[‡] For further literature on the vitamins see Sherman and Smith: " The Vitamins," Amer. Chem. Soc. Monograph Series, New York, 1922.

INDEX.